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DATE OF DEPOSIT: June 19, 2001

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FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. § 371		5585-59112
		U.S. APPLICATION NO. (If known, see 37 C.F.R. § 1.5) 09/868605
INTERNATIONAL APPLICATION NO. PCT/GB99/04200	INTERNATIONAL FILING DATE 17 December 1999	PRIORITY DATE CLAIMED 19 December 1998

TITLE OF INVENTION
IMPROVEMENT OF TOLERANCE TO A XENOGRAFT

APPLICANT(S) FOR DO/EO/US
Robert Ian Lechler, Nichola Jane Rogers, Anthony Dorling

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. § 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. § 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. § 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. § 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. § 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. § 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. § 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. § 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. § 371(c)(4)). (UNSIGNED)
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. § 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. §§ 3.28 and 3.31 and the Recordal fee of $40.00 is included.
13. ☒ A **FIRST** preliminary amendment.
    ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    ☒ Sequence Listing.
    ☒ Statement in Compliance.
    ☒ Computer readable form (diskette).
    ☒ Copy of International Search Report with cited references (see IDS).

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EXPRESS MAIL LABEL NO. EL828141257US
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U.S. APPLICATION NO. (If known, see 37 C.F.R. § 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/868605	PCT/GB99/04200	5585-59112

17. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 C.F.R. §§ 1.492(a)(1)-(5)):

	CALCULATIONS (PTO USE ONLY)
Neither International Preliminary Examination fee (37 C.F.R. § 1.482) nor International Search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO..... **$1,000.00**	
International Preliminary Examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO..... **$860.00**	
International Preliminary Examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO..... **$710.00**	
International Preliminary Examination fee paid to USPTO (37 C.F.R. § 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... **$690.00**	
International Preliminary Examination fee paid to USPTO (37 C.F.R. § 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)..... **$100.00**	

ENTER APPROPRIATE BASIC FEE AMOUNT =				$	860.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(e)).				$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	26 - 20 =	6	x **$18.00**	$	108.00
Independent Claims	2 - 3 =	0	x **$80.00**	$	0.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$270.00**	$	
TOTAL OF ABOVE CALCULATIONS =				$	968.00
☒ Reduction of 1/2 for filing by small entity. Small entity status is claimed for this application.				$	484.00
SUBTOTAL =				$	484.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 Months from the earliest claimed priority date (37 C.F.R. §§ 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$	484.00
Fee for recording the enclosed assignment (37 C.F.R. § 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. §§ 3.28, 3.31). **$40.00** per property. +				$	
TOTAL FEES ENCLOSED =				$	484.00
				REFUND →	$
				CHARGE →	$

a. ☒ A check in the amount of $ 484.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Director is hereby authorized to charge any additional fees that may be required, or credit any overpayment, to Deposit Account No. 02-4550. A duplicate copy of this sheet is enclosed.

d. ☒ Please return the enclosed postcard to confirm that the items listed above have been received.

NOTE: Where an appropriate time limit under 37 C.F.R. § 1.494 or § 1.495 has not been met, a petition to revive (37 C.F.R. § 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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cc: Docketing

09/868605

Attorney Reference Number 5585-59112
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PATENT

JC18 Rec'd PCT/PTO 1 9 JUN 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Lechler

Application No.

Filed: Herewith

For: IMPROVEMENT OF TOLERANCE TO A XENOGRAFT

Examiner:

Date: June 19, 2001

Art Unit:

CERTIFICATE OF MAILING

I hereby certify that this paper and the documents referred to as being attached or enclosed herewith are being deposited with the United States Postal Service on June 19, 2001 as Express Mail No. EL828141257US in an envelope addressed to: BOX PCT, COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231.

William D. Noonan

William D. Noonan, M.D., Attorney for Applicant

BOX PCT
COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

PRELIMINARY AMENDMENT

Before calculating the filing fee for the present application, please amend the claims as follows:

1. (Amended) A method of improving tolerance to a xenograft comprising: immunising a mammal with an immunogen comprising at least one T-cell epitope and at least one porcine polypeptide B-cell epitope, wherein said B-cell epitope is capable of mediating rejection of said xenograft.

2. (Amended) A method according to Claim 1, wherein said B-cell epitope is a peptide derived from at least one porcine polypeptide selected from the group of CD40, CD80, CD86 and VCAM.

3. (Amended) A method according to Claim 1, wherein said peptide is selected from at least one peptide represented in Figure 22.

4. (Amended) A method according to Claim1, wherein said peptide is selected from at least one peptide represented in Figure 24.

5. (Amended) A method according to Claim1, wherein said peptide is selected from at least one peptide represented in Figure 26.

6. (Amended) A method according to Claim 1, wherein said T-cell epitope comprises a tetanus toxoid polypeptide.

7. (Amended) A composition comprising an immunogen characterised in that said immunogen comprises at least one B-cell epitope and at least one T-cell epitope wherein said B-cell epitope comprises a porcine epitope involved in mediating xenograft rejection.

8. (Amended) A composition according to Claim 7, wherein said porcine epitope comprises a porcine polypeptide expressed by vascular endothelial cells of said xenograft.

9. (Amended) A composition according to Claim 7, wherein said B-cell epitope is selected from the group of CD40, CD86, CD80 and VCAM.

10. (Amended) A composition according to Claim 9, wherein said B-cell comprises at least one peptide as represented in Figure 22.

11. (Amended) A composition according to Claim 9, wherein said B-cell epitope comprises at least one peptide as represented in Figure 24.

12. (Amended) A composition according to Claim 9, wherein said B-cell epitope comprises at least one peptide as represented in Figure 26.

13. (Amended) A composition according to Claim 9, wherein said B-cell epitope comprises an extracellular domain of CD86.

14. (Amended) A composition according to Claim 7, wherein said T-cell epitope comprises a tetanus toxoid epitope.

15. (Amended) A composition according to Claim 7, wherein said composition further comprises a carrier capable of enhancing the immune response to said immunogen.

16. (Amended) An antibody, or the effective part thereof, wherein said antibody is capable of distinguishing between porcine polypeptides according to Claim 7, and the homologous polypeptides of the mammal receiving said xenograft.

17. (Amended) An antibody according to Claim 16, wherein said antibody is monoclonal.

18. (Amended) An antibody according Claim 16, wherein said antibody is a modified antibody comprising at least one detectable label.

19. (Amended) A method to monitor an immune status of a mammalian recipient of a xenograft comprising:

i) removing a sample from a xenograft recipient to be tested;

ii) contacting said sample to the antibody according to Claim 16; and

iii) monitoring expression of a porcine polypeptide shown in Figures 22, 24, or 26.

20. (Amended) A method of treating a mammal prior to receiving a xenograft, comprising:

i) immunising a mammal with an immunogenic composition according to Claim 7;

ii) assessing an immune status of said mammal to said immunogenic composition;

iii) transplanting said xenograft tissue/organ into a recipient mammal; and

iv) monitoring a rejection response to said xenograft.

21. (Amended) A method according to Claim 20, wherein said xenograft is of porcine origin and said mammal is human.

22. (Amended) A method according to Claim 20, wherein said xenograft comprises at least one vascularised graft and/or immunogenic porcine cell/tissue.

23. (Amended) A method according to Claim 20, wherein said xenograft comprises pancreatic islets.

24. (New) The method Claim 1, wherein said B-cell epitope has less than 75% sequence identity to a corresponding region of an equivalent human polypeptide.

25. (New) The method of Claim 7, wherein said B-cell epitope has less than 75% sequence identity to a corresponding region of an equivalent human polypeptide.

26. (New) The method of Claim 16, wherein said B-cell epitope has less than 75% sequence identity to a corresponding region of an equivalent human polypeptide.

REMARKS

The claims in this application have been amended, solely for the purpose of complying with U.S. claiming conventions.

Respectfully submitted,

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Lechler et al.

Art Unit:

Application No.

Filed: Herewith

For: IMPROVEMENT OF TOLERANCE TO A XENOGRAFT

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William D Noonan

William D. Noonan, M.D., Attorney for Applicant

STATEMENT IN COMPLIANCE WITH 37 C.F.R. § 1.821(f)

BOX PCT
COMMISSIONER FOR PATENTS
Washington, DC 20231

Sir:

In compliance with 37 C.F.R. § 1.821(f), the undersigned declares that the nucleotide and/or amino acid sequences presented in the paper copy of the "Sequence Listing" submitted herewith are the same as the sequences contained in the computer-readable form of said "Sequence Listing." No new matter has been added.

Respectfully submitted,

KLARQUIST SPARKMAN CAMPBELL
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Marked-up Version of Amended Claims
Pursuant to 37 C.F.R. §§ 1.121(b)-(c)

CLAIMS

1. A method of improving tolerance to a xenograft comprising[;] : immunising a mammal with an immunogen comprising at least one T-cell epitope and at least one porcine polypeptide B-cell epitope, [characterised in that] wherein said B-cell epitope is [derived from at least one porcine polypeptide involved in] capable of mediating [the] rejection of said xenograft.

2. A method according to Claim 1, [characterised in that] wherein said B-cell epitope is a peptide derived from at least one porcine polypeptide selected from[;] the group of CD40[;] , CD80[;] , CD86 [or] and VCAM.

3. A method according to Claim 1, [or 2 characterised in that] wherein said peptide is selected from at least one peptide represented in Figure 22.

4. A method according to Claim1, [or 2 characterised in that] wherein said peptide is selected from at least one peptide represented in Figure 24.

5. A method according to Claim1, [or 2 characterised in that] wherein said peptide is selected from at least one peptide represented in Figure 26.

6. A method according to [Claims 1 - 5 characterised in that] Claim 1, wherein said T-cell epitope [is derived from] comprises a tetanus toxoid polypeptide.

7. A composition comprising an immunogen characterised in that said immunogen [has] comprises at least one B-cell epitope and at least one T-cell epitope wherein said B-cell epitope [is derived from at least one] comprises a porcine [polypeptide] epitope involved in mediating xenograft rejection.

8. A composition according to Claim 7, [characterised in that] wherein said porcine epitope comprises a porcine polypeptide [is] expressed by vascular endothelial cells of said xenograft.

9. A composition according to [Claims 7 or 8 characterised in that] Claim 7, wherein said B-cell epitope is [derived from at least one porcine polypeptide] selected from[;] the group of CD40[;] , CD86[;] , CD80[;] and VCAM.

10. A composition according to Claim 9, [characterised in that] wherein said B-cell epitope [is selected from] comprises at least one peptide as represented in Figure 22.

11. A composition according to Claim 9, [characterised in that] wherein said B-cell epitope [is selected from] comprises at least one peptide as represented in Figure 24.

12. A composition according to Claim 9, [characterised in that] wherein said B-cell epitope [is selected from] comprises at least one peptide as represented in Figure 26.

13. A composition according to [Claims 9 or 12 characterised in that] Claim 9, wherein said B-cell epitope [is derived from the] comprises an extracellular domain of CD86.

14. A composition according to [Claims 7 - 13 characterised in that] Claim 7, wherein said T-cell epitope [is derived from] comprises a tetanus toxoid epitope.

15. A composition according to [Claims 7 - 14 characterised in that] Claim 7, wherein said composition further comprises a carrier capable of enhancing the immune response to said immunogen.

16. An antibody, or the effective part thereof, [characterised in that] wherein said antibody is capable of distinguishing between porcine polypeptides according to [Claims 7 – 15] Claim 7, and the homologous polypeptides of the mammal receiving said xenograft.

17. An antibody according to Claim 16, [characterised in that] wherein said antibody is monoclonal.

18. An antibody according to [Claims 16 or 17 characterised in that] Claim 16, wherein said antibody is a modified [with] antibody comprising at least one detectable label.

19. A method to monitor [the] an immune status of a mammalian recipient of a xenograft comprising:

iii) removing a sample from a xenograft recipient to be tested;

iv) contacting said sample to the antibody according to [Claims 16 – 18] Claim 16; and

iii) monitoring [the] expression of [the] a porcine polypeptide [according to Claims 4 –8] shown in Figures 22, 24, or 26.

20. A method [to treat] of treating a mammal prior to receiving a xenograft, comprising:

i) immunising a mammal with an immunogenic composition according to [Claims 7 – 15] Claim 7;

ii) assessing [the] an immune status of said mammal to said immunogenic composition;

iii) [transplantation of] transplanting said xenograft tissue/organ into a recipient mammal; and

iv) monitoring [the] a rejection response to said xenograft.

21. A method according to Claim 20, [characterised in that] wherein said xenograft is of porcine origin and said mammal is human.

22. A method according to Claim 20, [or 21 characterised in that] wherein said xenograft [is] comprises at least one vascularised graft and/or immunogenic porcine cell/tissue.

23. A method according to Claim 20, [characterised in that] wherein said xenograft [is] comprises pancreatic islets.

24. (New) The method Claim 1, wherein said B-cell epitope has less than 75% sequence identity to a corresponding region of an equivalent human polypeptide.

25. (New) The method of Claim 7, wherein said B-cell epitope has less than 75% sequence identity to a corresponding region of an equivalent human polypeptide.

26. (New) The method of Claim 16, wherein said B-cell epitope has less than 75% sequence identity to a corresponding region of an equivalent human polypeptide.

IMMUNOSUPPRESSION

1. FIELD OF THE INVENTION

This invention relates to immunosuppression and, more particularly, to immunosuppression in the context of xenotransplantation.

2. BACKGROUND TO THE INVENTION

Despite the established success of allogeneic organ transplantation, the increasing disparity between the supply and demand of organs must be overcome. Increasing the supply of allogeneic organs does not offer a satisfactory solution because even if all usable organs were transplanted this would still not meet the existing demand (1,2). This has led to a resurgence of interest in xenotransplantation (the transplantation of organs between animals of different species) as a viable and attractive alternative.

Xenotransplantation research has recently focused on the pig as a suitable animal donor in terms of size, physiological compatibility and breeding characteristics (3,4). Until recently however , discordant xenotransplantation has been limited by the inevitable occurrence of humorally-mediated hyperacute rejection (HAR) which rapidly triggers organ rejection upon revascularisation. HAR is the fate of most organs transplanted between discordant species. Recently, significant advances have been made in understanding the immunological basis of HAR, and many approaches have been employed to overcome it. Of significance, a variety of transgenic strategies are currently being employed including the expression of regulators of complement activity on porcine endothelial cells (5). It is foreseeable that short-term xenograft survival will soon be achieved (6). The recent advances in overcoming HAR have highlighted subsequent immunological barriers which must be surmounted to enable long-term xenograft survival. Both humoral and cellular arms of the immune response appear to play a role in the downstream events of immunological rejection. Clearly the most important of which is the existence of a formidable T cell mediated rejection response (7-11) previously obscured by the dominant role of HAR. *In vitro* , human T cells have been demonstrated

to play a central role in the recognition of xenogeneic cells (7,8,12) following sensitisation via the direct and indirect T cell activation pathways, which have been well documented for allorecognition and allograft rejection (13). Knowledge of the cellular mechanisms underlying allorejection has provided an important basis for the investigation of the T cell mediated xenoresponse.

At present, the major therapies to prevent cell mediated rejection of organ transplants rely on systemic immunonosuppressive drugs or monoclonal antibody (Mab) therapy directed against targets such as CD3, CD4, CD25, (14). Following reports that strong T cell xenoresponses can be generated *in vitro* (7,8,12), control of xenograft rejection may require levels of immunosuppresion much greater than the current standard doses. Such a strategy would not be desired in a xenograft context. Drugs must be taken for life, depress the entire immune system and result in an increased risk of infection and susceptibility to cancer (14). For the applicability of xenotransplantation to the clinic, targeting graft-specific strategies for tolerance induction/immunosuppression would clearly be highly advantageous. Whilst this has been difficult to achieve in an allotransplant context, xenotransplantation offers greater potential - with differences between species providing the option for the generation of reagents that are truly graft specific. In addition, there is the opportunity for the manipulation of both the porcine donor organ, and the human recipient's immune system, prior to transplantation (1).

3. DETAILED BACKGROUND

3.1 T cell activation and proliferation

Optimal proliferation of T cells, although initiated via ligation of the antigen specific CD3/TCR complex (Signal 1) requires additional costimulatory signals (Signal 2) (15,16,17) which are usually supplied by the antigen presenting cell (APC). Whilst antigenic stimulation of T cells in the presence of signal 2 induces T cell activation and proliferation (18), exposure of T cells to MHC-antigen complexes in their absence leads to aborted T cell proliferation and the development of clonal anergy (19,20). Manipulation of APC by aldehyde fixation (20,21) or heat treatment (19) has been

demonstrated to abrogate the ability of such cells to activate alloreactive T cells, without altering levels of MHC-II surface expression. Thus T cell receptor occupancy alone is insufficient to fully activate the T cell (17). Anergic T cells are best characterised by their lack of IL-2 production and their continued inability to produce IL-2 on subsequent exposure to antigen (22). Thus, confirming the two signal model of activation as predicted by Lafferty *et al* (23). For T cells to respond to a given antigenic stimulus, multiple activation signals are required from the APC (23).

The *in vivo* induction of T cell anergy in the absence of a secondary signal was first demonstrated by Jenkins and Schwartz in 1986 (24) using chemically fixed APC to present specific peptide to CD4 T helper clones. A multitude of *in vitro* and *in vivo* data has since been produced supporting the hypothesis that signal 1 in isolation fails to activate T cells (22), and that costimulatory signalling results from contact with other cells rather than via soluble factors. Fibroblasts transfected with human Class II MHC molecules, but not expressing the appropriate CS signals (lacking signal 2) can efficiently present antigen to class II restricted CD4 T cell clones, but these fail to cause antigen specific T cell proliferation, rendering cells anergic. The context in which T cells first encounter antigen therefore has an important bearing on subsequent immune responsiveness.

Thus, costimulatory molecules are essential for T cell activation and multiplication and result from interactions between receptors on T cells and their ligands expressed on the APC. The costimulatory signal itself, however, is neither antigen specific nor MHC restricted (25). In recent years the molecular interactions involved in mediating costimulation have been well defined. The two key pathways involve (i) B7-1, B7-2 (members of the B7 family) and (ii) CD40, which are expressed on the APC, and their counter-receptors CD28 and CD40 ligand (CD40L) respectively expressed on T cells. A large body of evidence, both *in vivo* and *in vitro* , clearly defines the crucial roles played by B7-1, B7-2 and CD40 in providing T cell costimulation (26-36). Furthermore, the simultaneous blockade of signalling via CD28-B7 and CD40-CD40L in an allotransplant

context prevented the onset of allograft rejection (37,38). *In vivo* , targeting the B7/CD28 interaction has been shown to prevent T cell sensitisation to graft antigen, thereby prolonging graft survival (38,39).

T cells can be sensitised against xenoantigens via one of two pathways - the direct and indirect pathways, which are analogous to the well documented T cell activation pathways against alloantigens (Figure 1). Direct recognition requires that the recipient T cells recognise intact xeno MHC-molecules complexed with peptide on donor stimulator cells. In contrast, indirect recognition requires that recipient APC process the xenoantigen prior to presentation to recipient T cells in the context of recipient MHC II. Self MHC II restricted T cells with specificity for the xenoantigen will recognise the peptide and respond. Whilst the majority of data reported is of indirect xenorecognition responses, cell mediated rejection via the direct route has also been documented (7,8,9,11,12,40,41,42). Vigorous human T cell proliferative responses directed against porcine tissues *in vitro* have been documented from studies both in this laboratory and others.

3.2 Costimulatory molecules

The crucial role played by costimulatory molecules in determining the result of TCR-CD3 receptor engagement with MHC and peptides has been demonstrated extensively both *in vivo* and *in vitro* . Anti-costimulatory molecule strategies aimed at either the receptors or their ligands are being used as therapeutic strategies for altering the immune response. Such approaches have been tested in model transplant systems to alter cell mediated responses thereby preventing graft rejection (14,37,38,43-47).

B7-1 (B7/BB1, CD80) and B7-2 (CD86) both belong to the immunoglobulin superfamily and are heavily glycosylated transmembrane proteins (25). B7-1, a B cell activation molecule was first identified in 1989 (27), followed by B7-2 in 1993 (49). Both human B7-1 and B7-2, and the murine homologues have now been cloned and functionally characterised (25) . B7-1 and B7-2 are constitutively expressed on splenic and blood

dendritic cells and are induced on B cells and monocytes upon activation (34,50,). B7-1 and 2 are highly homologous and are the natural ligands for the T cell antigen CD28 (50). Cytotoxic T lymphocyte antigen-4 (CTLA-4), a cell surface glycoprotein has been identified as a second receptor for the B7 family of molecules (51) and is homologous to CD28 with 31% sequence identity. Both B7 isoforms bind to CTLA-4 with higher affinity than to CD28 (30,50,52). Whilst CD28-B7 receptor engagement results in an APC-derived costimulatory signal involved in antigen specific IL-2 production both *in vivo* and *in vitro* (53,54), CTLA4 appears to function as a negative regulator of T cell activation (55, 56, 57). Cross-linking by anti-CTLA4 antibodies has been demonstrated to antagonise CD28 ligation (58) and, in addition, CTLA4 knock-out mice die due to uncontrolled lymphocyte proliferation within the first few weeks of life (59). Thus, CTLA4 ligation is thought to be crucial for the maintenance and regulation of immune responses. The underlying mechanisms have not, however, been clearly defined.

Among costimulatory molecules, the B7 family appears to be unique, since ligation by CD28 of either B7-1 or B7-2 is both necessary and sufficient to prevent the induction of anergy (34). The CD28-B7 interaction is thought to deliver crucial signals to sustain proliferation of activated T cells. These observations are supported by *in vitro* data showing that whilst cells deficient in B7 fail to stimulate a primary MLR, transfectants expressing high levels of B7 gained the capacity to stimulate the production of IL-2 by alloreactve T cells and to co-stimulate a polyclonal population of purified T cells cultured with immobilised anti-CD3 Mab (31). Artificial APC generated by stably transfecting NIH-3T3 cells with HLA-DR7, B7 or both, clearly demonstrated that following presentation of tetanus toxoid (TT) optimal T cell proliferation and IL-2 production resulted only when both molecules were present. In the absence of B7, clonal anergy resulted (58).

Porcine B7-2 (PoB7-2) has been cloned from aortic endothelial cells (60). Following transient transfection of porcine B7-2, human umbilical vein endothelial cells strongly costimulated IL-2 production by human T cells. This costimulation of human T cells by

poB7-2 was shown to be as effective as costimulatory signals provided by human B7-1 or B7-2 and could be specifically blocked by huCTLA4Ig. Thus poB7-2 strongly contributes to the immunogenicity of porcine endothelium (60).

Although B7-1 and B7-2 mediated interactions appear to be central to the development of T cell specific immunity, additional costimulatory pathways of importance exist. The most crucial of which involves the CD40 and CD40 ligand (CD40L) interaction (34).

CD40 is a 50kDa surface glycoprotein belonging to the TNF-receptor superfamily. CD40 is expressed on various APC including among others, monocytes, dendritic cells and activated macrophages. Other cell types including endothelium also express CD40 (34). Its counter-receptor CD40L (CD154, gp39, TRAP) is a 33 kDa type II integral membrane protein (34,36) transiently expressed on activated CD4 T cells. The CD40-CD40L interaction has been demonstrated to play an important role in both the humoral and cellular arms of the immune response with a dominant role in B cell activation. Whilst cross linking of CD40 on B cells is essential for B cell growth and isotype switching, it also results in the upregulation of B7 expression (50). Levels of B7 expression (and thus APC capacity) of monocytes and dendritic cells are clearly unregulated following CD40 signalling (34). Data from CD40 knock-out mice demonstrated that CD40L signalling following ligation by CD40 plays an important role in T cell activation (61). Transfection of the murine P815 mastocytoma cells with CD40 (or B7-1) enabled previously non-stimulatory P815 cells to mediate the costimulation necessary for polyclonal T cell activation and the generation of cytokines (34). CD40-CD40L interactions have also been demonstrated to play a critical role in allograft rejection (62,63).

Resting B cells do not normally express B7-1/B7-2 at high levels until they are activated (50). Activation of B cells following simultaneous engagement of MHC-peptide/TCR and CD40-CD40L leads to the upregulation of B7 family members on B cells, thereby enhancing the stimulation and subsequent activation of T cells (34,36). Thus, the CD40-CD40L interaction influences costimulatory activity by inducing expression of the

B7 family of molecules and perhaps other costimulatory molecules, thereby playing a key role in T cell activation . The clear synergistic effects of CD40 and B7 indicate the importance of both costimulatory pathways for the initiation and amplification of T cell dependent immune responses (38). CD40-CD40L interactions have also been shown to play a crucial role in the generation of cytotoxic T lymphocyte (CTL) responses by modifying the functional status of a dendritic cell (64,65,66)

Extensive studies have demonstrated the importance of blocking B7-CD28 and/or CD40-CD40L interactions in the context of both allo and xenotransplantation. Data strongly supporting this includes the use of CTLA4Ig to block signalling via CD28-B7 resulting in enhanced graft survival and the prevention of chronic rejection in a rat cardiac allograft model (44,45) and a murine aortic allograft model (43). In these models, administration of CTLA4Ig caused partial (44) or complete (46) tolerance to graft antigen by inducing T cell anergy. Treatment of allo pancreatic islet transplants with anti-B7-2 and B7-1 antibody has also been demonstrated to inhibit transplant rejection (14). Similar results were obtained in models inhibiting CD40 signalling in a mouse cardiac allotransplant models (37,47,62). Two studies detailing the simultaneous blockade of signalling via CD28-B7 and CD40-CD40L prevented the onset of allorejection. Concurrent prolonged inhibition of both pathways completely abrogated the onset of chronic rejection in a mouse allo model (37) and in a skin and heart allo model (38).

In the realm of xenotransplantation, Lenshow and colleagues have, demonstrated long-term donor specific tolerance of human islets transplanted into mice with concomitant treatment with CTLA4Ig (46). Graft specific tolerance was demonstrated to be a direct consequence of inhibiting recognition via B7 expressing APC. In addition, Tran *et al* (67) demonstrated short term suppression with CTLA4-Fc treatment. There is limited data available on the simultaneous blockade of both pathways in the xenotransplantation context, with the prolonged survival of rat and porcine skin transplanted into murine recipients (63).

In vitro and *in vivo* data have clearly demonstrated that targeting the interactions mediated by either the CD28-B7, CD40-CD40L, or both pathways has prevented the sensitisation of T cells to alloantigen and xenoantigen from engrafted tissue thereby prolonging graft survival ().

As noted above, T- cell mediated graft rejection is well documented. The immune system can mount alternate or additional cell mediated rejection mechanisms. These mechanisms are illustrated by the function of various molecules expressed by, *inter alia*, endothelial cells. VCAM is a cell adhesion molecule, expressed by endothelial cells, that is thought to have a role in leukocyte recruitment to sites of inflammation. VCAM is an inducible transmembrane glycoprotein which has a basal level expression in resting endothelial cells but is rapidly expressed upon exposure to pro-inflammmatory cytokines (eg IL-1, TNFα). The interaction of VCAM with leukocytes is via the very late antigen 4 (VLA-4) expressed at the leukocyte cell surface. Therefore endothelial cell expression of VCAM functions to induce the infiltration of VLA-4 presenting leukocytes to sites of inflammation which augments rejection responses to allografts or xenografts.

It is believed that porcine VCAM plays an important role in allowing the migration of human leukocytes across porcine endothelial cell monolayers. There is a rationale for believing that blocking this interaction will have beneficial consequences on xenograft survival. Pig VCAM, cloned in 1994, has significant homology with human VCAM(1). As well as the data presented in (1), there is a wealth of evidence from other in vitro studies suggesting that pig VCAM interacts efficiently with human leukocyte- expression counter receptor, VLA-4. For instance, in static adhesion assays, antibodies to VCAM significantly inhibit the binding of human NK and T cells to pig endothelium. With NK cells, this disruption inhibits cell lysis which normally results after adhesion to porcine endothelial monolayers.

The effect of anti-VCAM antibodies on T cell mediated xenograft rejection mechanisms is more difficult to predict. In some rodent models of allotransplantation, antibodies

against VCAM have been used to prolong allograft survival. In some instances, long term survival and specific tolerance have been described (2,3), although the precise mechanism of action of these studies was not fully elucidated.

3.5 Peptide immunisation strategy

Previous *in vivo* studies using synthetic peptides conjugated to carrier molecules as immunogens have demonstrated the ability to generate the production of biologically active antibodies (68). There is now an extensive literature detailing peptide immunisation strategies which demonstrate enhancement of antibody production by carrier presentation(68-72). Thus, appropriate T cell epitopes can be used to prime T cells for subsequent help to B cells. Recent data has been published reporting the production of IgG by self-reactive B cells following immunisation with a self reacting antigen covalently coupled to a carrier molecule (70). Thereby demonstrating that B cell tolerance to self protein can be overcome.

As mentioned above, in order to be recognised by T cells, antigen (self or foreign) must be processed and presented by APC. B cells can act as highly potent APC following endocytosis of antigen via IgG receptors . In the presence of a full complement of activation signals (TCR engagement plus costimulation) T cell activation will occur resulting in the subsequent generation of antibody.

Peptides from self proteins are processed and presented to T cells in the same manner as foreign proteins, but because of T cell tolerance, presentation of self peptides does not normally result in T cell activation (70). The absence of T cell recognition may therefore explain, in part, why potentially reactive B cells fail to respond.

The ability to overcome B cell non-responsiveness to self peptides has recently been demonstrated by Dalum *et al* (69). An autoantibody response was generated by the provision of additional T cell help in the form of a strong foreign carrier T cell epitope. Further studies have demonstrated that synthetic peptides conjugated to T cell carrier

molecules are capable of overcoming B cell non-responsiveness if significant numbers of self-reactive B cells are present in the host (69,70). Insertion of a single foreign T cell epitope into the sequence of Ubiquitin, elicited strong autoantibody production directed against the native molecule (69). In an elegant study by Sad, using GnRH as a self protein chemically linked to diphtheria toxoid (DT) as the synthetic T cell epitope, autoantibodies were produced with specificity for native GnRH (71,72). Following the initial vaccination, the continued presence of the native GnRH *in vivo* maintained the production of Ab. Continued antibody production caused sterility in the immunised mice due to the sustained anti-GnRH antibody response maintained by the continued presence of the native molecule against which the specific B cells were producing antibody. The DT carrier provoked a helper T cell response to assist GnRH specific B cells and break B cell tolerance.

4. STATEMENTS OF INVENTION

In its broadest aspect the invention relates to the immunisation of a mammal, preferably a human, with an immunogen which results in the production of antibodies specific to porcine epitopes expressed, typically, but not exclusively, by porcine endothelial cells which are involved in mediating xenograft tissue/organ immune rejection.

Immunogen is herein construed as any epitope or combination of epitopes capable of invoking an immune response. The epitope may be T cell specific or B- cell specific. In this context, epitope is construed as any polypeptide, peptide, modified polypeptide, modified peptide (eg typically modification may be by glycosylation or phosphorylation of the epitope).

Typically, the invention encompasses epitopes derived from porcine molecules which are selected from at least one of: CD40; B7.1; B7.2; VCAM.

It will be apparent to one skilled in the art that the invention provides means to immunise an individual, ideally prior to xenotransplantation, with an immunogen to a part of a

porcine molecule which contains a B-cell epitope not present in the homologous mammalian polypeptide to ensure the selective production of antibodies to the porcine polypeptide without the development of antibodies to the patients own functional equivalent and without the development of CD4 T cell responses thereby avoiding cell mediated rejection. In addition the immunogen provides blocking antibodies generated by the recipient which abrogate the activity of porcine polypeptides which mediate a rejection response.

It will be still further apparent to one skilled in the art that the invention has significant advantages over prior art attempts to immunosupress a recipients immune system to porcine cells/tissues. For example, WO 97119971 discloses the use of B7.2 or VCAM polypeptides to produce diagnostic and therapeuctic antibodies to monitor transplantation rejection and to block xenotransplant rejection.

This has significant disadvantages. The treatment of a transplant patient with an antibody to, for example VCAM or B7.2, requires periodic administration throughout the life of the patient to maintain the blocking properties of the antibody. Moreover, the immune system will ultimately raise antibodies to the therapeutic antibodies (anti-idiotypic antibodies)resulting in their removal from the patients circulation.

The present invention does not require periodic administration since it is the patients own immune system that is responsible for the production of blocking antibodies to porcine polypeptides. The immune system will not recognise these antibodies as foreign and will therefore not result in the production of anti-idiotypic antibodies.

The present invention involves the use of a foreign T cell epitope to exert significant influences on subsequent responses to molecules conjugated to the carrier. By such means autoantibody responses may be directed against porcine polypeptides in a xenotransplantation context.

According to the present invention there is provided a method of improving the tolerance of an animal, including a human being, to a xenograft, the animal having T cell mediated immunity, the method comprising causing the animal to raise an antibody against a xeno-molecule involved in the generation of a rejection response in the animal, said antibody being raised by immunising the animal with a chimeric peptide comprising a T cell epitope against which the animal has immunity and a B cell epitope of said xenomolecule.

Accordingly, xenograft specific tolerance is induced in transplant recipients by targeting the direct T cell mediated response by the use of chimeric peptide constructs to stimulate the generation of specific anti-graft tolerance-promoting antibodies by the recipient prior to transplantation. By way of example, the chimeric peptides comprise a T cell epitope conjugated to sequences of porcine polypeptides, B7-1, B7-2, CD40, VCAM. The presence of the engrafted tissue will then serve to maintain and perpetuate the production of antibody by the recipient's B cells.

The present invention also provide a chimeric peptide comprising a T cell epitope and a B cell epitope, said T cell being that of an animal, including a human being of a first species and said B cell being of an animal of a second species, said first and second species such that xeno transplantations suitable from an animal of said second species to an animal of said first species.

In addition, the present invention provides the use of a chimeric peptide improving the tolerance of an animal, including a human being, to a xenograft, the chimeric peptide being as defined above.

According to a further aspect of the invention said immunogenic composition comprises at least one T- cell epitope and at least one B- cell epitope characterised in that said B – cell epitope is derived from at least one porcine polypeptide involved in mediating

xenograft rejection and said T cell epitope is derived from a molecule to which the recipient is already immune.

In yet a further preferred embodiment of the invention said immunogenic composition comprises at least one peptide antigen derived from at least one of porcine: CD40; VCAM; CD86; CD80.

Preferably said peptide antigen is derived from porcine CD40. Ideally said peptide is derived from the amino- terminal domain of porcine CD40, or at least that part of the amino terminal domain that is exposed at the cell surface of a porcine cell presenting CD40. More ideally still said peptide antigen is selected from the peptide sequences presented in Figure 22

Preferably said peptide antigen is derived from porcine VCAM. Ideally said peptide is derived from the amino- terminal domain of porcine VCAM, or at least that part of the amino terminal domain that is exposed at the cell surface of a porcine cell presenting VCAM. More ideally still said peptide antigen is selected from the peptide sequences presented in Figure 24

Preferably said peptide antigen is derived from porcine CD86. Ideally said peptide is derived from the amino- terminal domain of porcine CD86, or at least that part of the amino terminal domain that is exposed at the cell surface of a porcine cell presenting CD86. More ideally still said peptide antigen is selected from the peptide sequences presented in Figure 26.

Preferably, said peptide antigen comprises at least 9 amino acid residues. More ideally still said peptide comprises 10 – 30 amino acid residues.

According to a further aspect of the invention there is provided an immunogenic composition according to any previous aspect or embodiment of the invention wherein

said composition further comprises at least one agent capable of enhancing the immune response to said immunogenic composition.

In a preferred embodiment of the invention said agent is a carrier / adjuvant.

It is well known in the art that carriers/adjuvants are useful in promoting immune responses to selected antigens. These adjuvants are either crosslinked or coupled to the antigen or co-administered to the animal with the antigen. Adjuvants useful in promoting immune responses are detailed in Vaccine Design:The Subunit and Adjuvant Approach Chapter 7, p141- 228, Plenum Press, New York, 1995. Various carriers, excipients or diluants are available in which said immunogenic composition can be stored and/or administered. For example, and not by way of limitation, the encapsulation of the immunogenic composition in liposomes is a conventional practice. Liposomes are phospholipid based vesicles which are useful as carrying agents for immunogenic compositions and the like.

According to yet a further aspect of the invention there is provided an antibody, or at least the effective part thereof, directed to at least one region of at least one porcine polypeptide according to the invention.

In a preferred embodiment of the invention said antibody is a monoclonal antibody, or at least the effective part thereof. Ideally said antibody is labelled.

It will be apparent to one skilled in the art that antibodies according to the invention will have utility with respect to monitoring the expression of porcine polypeptides presented by porcine tissues/organs.

According to a further aspect of the invention there is provided a method to monitor the immune status of a mammalian recipient of a xenograft. Preferably said monitoring method is *in vitro*.

According to yet a further aspect of the invention there is provided a method to improve the tolerance of an animal to a xenograft comprising:

i) administering at least one immunogenic composition according to any previous aspect or embodiment of the invention to an animal; optionally

ii) monitoring the immune status of said animal to said immunogenic composition;

iii) transplantation of at least one porcine tissue/organ into said animal; and, optionally

iv) monitoring the animal for a rejection response to said porcine tissue/organ.

In a preferred method of the invention said animal is human.

In a further preferred method of the invention said xenograft is any vascularised graft and/or immunogenic porcine cell/tissue.

In a further preferred method of the invention said xenograft is porcine pancreatic islets.

It will be apparent to one skilled in the art that (ii) above can be conducted either by monitoring for the presence of antibodies to co-stimulatory molecules in sera (for example by ELISA or by FACS analysis of cells expressing said co-stimulatory molecules), or alternatively, or in addition, monitoring the presence of cytolytic T- cells in the blood of the treated animal by conventional T- cells lysis assays.

The potential benefits of the use of a chimeric peptide of the invention are that it avoids the need for injection of blocking antibodies or fusion proteins. Furthermore, the induction of a recipient antibody response circumvents the problems most commonly associated with administration of xenogeneic antibodies or fusions proteins, namely the immune response against the administered reagent.

An embodiment of the invention will now be described, by example only and with reference to the following Tables and Figures;

Table 1 represents the regions of non-homology in human CD40 with respect to the homologous porcine CD40;

Table 2 represents the regions of non- homology in human VCAM with respect to the homologous porcine VCAM;

Table 3 represents the regions of non-homology in human CD86 with respect to the homologous porcine CD86;

Figure 1a is a diagrammatic representation of direct xenorecognition and Figure 1b is a diagrammatic representation of indirect xenorecognition;

Figure 2 represents the porcine CD86 nucleic acid sequence;

Figure 3 represents the porcine CD86 cDNA sequence obtained by reverse transcription of porcine mRNA followed by PCR amplification;

Figure 4 represents a comparison of the nucleotide sequence of the cDNA in Figure 2 with the published porcine CD86 sequence;

Figure 5 represents a comparison of the cDNA sequence in Figure 2 with the published murine and human CD86 sequences;

Figure 6 represents the translated amino acid sequence of the cDNA in Figure 2 compared with porcine, human and murine amino acid sequences;

Figure 7 represents the position of porcine B7.1 oligonucleotide primers with respect to the human and murine B7.1 nucleic acid sequences;

Figure 8a represents a comparison of the human, murine and bovine CD40 nucleic acid sequences; Figure 8b represents a comparison of the human, murine and bovine CD40 amino acid sequences;

Figure 9 represents FACS analysis of the expression of CD86 (B7.2) after transfection with a vector encoding porcine CD86 (B7.2);

Figure 10 represents FACS analysis of the expression of CD86 (B7.2) by transiently transfected cells with a vector encoding porcine CD86(B7.2);

Figure 11 represents flow cytometric analysis of cells transfected with porcine CD86(B7.2);

Figure 12 represents the position of nine CD86(B7.2) derived peptides in the porcine CD86(B7.2) sequence;

Figure 13 represents a comparison of T cell proliferation response to whole ovalbumen or the ovalbumen peptide $Ova_{323-339}$;

Figure 14a represents the differential binding of B7.2 specific peptide sera or ovalbumen control sera by peptide ELISA;

Figure 14b represents the in vitro recognition of B7.2 derived peptides 4 and 6 by mouse sera immunised with peptides 4 or 6;

Figure 15a represents the in vitro recognition of the B7.2 peptide sera and control ova peptide sera by peptide ELISA;

Figure 15b represents the inhibition of direct mouse anti porcine T cell responses by peptide 4 and 6 sera which also shows no inhibition of of costimulation by murine CD86;

Figure 16 represents the differential binding of the B7.2 derived peptide 4 sera or ova control peptide sera by peptide ELISA;

Figure 17a represents flow cytometric analysis of P815 cells transfected with porcine CD86 following staining with sera from peptide 4 or control ova peptide sera;

Figure 17b represents FACS analysis of P815 cells transfected with porcine CD86 or CHO cells transfected with murine CD86 following staining with sera from mice sera derived from peptide 4 or peptide 6;

Figure 18 represents a preparation of porcine pancreatic islets isolated from a large white pig;

Figure 19 is a schematic representation of the chimeric peptide immunisation and transplantation protocol;

Figure 20 shows that anti-porcine CD86 antisera prolongs the survival of transplanted porcine pancreatic islets;

Figure 21 is a comparison of the amino acid sequence of porcine and human CD40 (underlined sequences are peptides identified in table 1);

Figure 22 is the translated amino acid sequence of porcine CD40 (underlined sequences are peptides identified in table 1);

Figure 23 is a comparison of the amino acid sequence of porcine and human VCAM (underlined sequences are peptides identified in table 2);

Figure 24 is the translated amino acid sequence of porcine VCAM (underlined sequences are peptides identified in table 2);

Figure 25 is a comparison of the amino acid sequence of porcine and human CD86 (underlined sequences are peptides identified in table 3); and

Figure 26 is the translated amino acid sequence of human CD86 (underlined sequences are peptides identified in table 3)

5. SPECIFIC EMBODIMENTS

5.1 Cloning porcine costimulatory molecules

5.1.1 Cloning porcine B7-2

RNA was extracted from primary and transformed porcine cells using a standard protocol. mRNA was then reverse transcribed and porcine B7-2 (poB7-2) amplified from the cDNA by 35 cycles of PCR at 56^0 C with 1.5mM magnesium. The 5' and 3' primers GCATGGATCCATGGGACTGAGTAACATTCTCTTTG and GCATGTCGACTTAAAAATCTGTAGTACTGTTGTC respectively were designed on the basis of the published poB7-2 sequence (60) to overlay the start and stop codons (Figure 2). A 956 base pair fragment was generated and subcloned into the BamH1 & Sal1 restriction sites of pbluescript. The nucleotide sequence was determined using standard m13 forward and reverse primers. The sequence of a single clone, CD86(i) is illustrated in Figure 3, with comparison to the published sequences from porcine (Figure 4), human and murine B7-2 (Figure 5). One base pair difference is detected between our clone, CD86(i), and the published sequence at the 3' prime end. This, however, is unlikely to be an important difference with respect to either poB7-2 expression or binding to its ligand. The predicted amino acid sequence of CD86(i) , compared to that of porcine, human and mouse B7-2 is shown in Figure 6.

5.1.2 Cloning porcine B7-1 and CD40

RNA extracted from phytohaemagglutinin (PHA) or poke-weed mitogen (PMW) stimulated porcine PBMC and transformed porcine endothelial cells is being used to amplify cDNA encoding the costimulatory molecules B7-1 and CD40. B7-1 Primers were designed on the basis of conserved areas following comparison of murine and human (29,49) sequences. External (lying outside the coding region) AGACCGTCTTCCTTTAG(3'i), TTGGATCCTCCATGTTATCCC (3'ii) and AGCATCTGAAGC (5') and internal (within the coding region) ATGGATCCTCCATTTTCCAACC (3') and TTGTCGACATCTACTGGC (5') primers have been designed as depicted in Figure 7. The generation of two 3' primers is due to significant differences between the human and murine sequences in the terminal coding regions. Resulting PCR fragments will be subcloned as described above using the restriction sites BamHI and SalI contained within the promoter sequence. Constructs will then be sent for sequence confirmation.

CD40 primers were designed in a similar manner following sequence alignment of published CD40 sequences from human, mice and cattle (73,74,75) as illustrated in Figures 8A & B. The 5' and 3' primer sequences are GGATCCTCACTGTCTCTCCTGCACTGAGATGCGACTCTCCTCTTTGCCGTCCG TCCTCC and GAATTCATGGTTCTGTTGCCTCTGCAGTG respectively containing the BamHI and EcoRI restriction sites.

5.2 Generation of porcine costimulatory molecule expressing cell transfectants

The poB7-2 molecule (CD869(i)) has been subcloned into the eukaryotic expression vector pci.neo carrying the neomycin drug-selectable marker. This is being used to transfect M1 and M1.DR1 transformed murine cell lines using a standard calcium phosphate precipitation method. G418 resistant pci.neo expressing cells will be selected using dynabead purification and highly expressing clones is selected by limiting dilution.

Stable poB7-2 M1 and P815 transfectants have been generated by this approach using the poB7-2 DNA construct supplied to us by Maher *et al* (Figure 9). transient transfections of M1 and P815 cells have been generated using our CD86(i) construct (Figure 10).

3 particular assays are undertaken using the CD86(i) transfected cells.

(I) comparative costimulatory function of poB7-2 with human B7-1 in the context of MHC restriction;

(II) flow cytometric analysis of specific anti-poB7-2 antibodies in the sera of immunised mice; and

(III) generation of specific anti-poB7-2 monoclonal antibodies.

(I) Comparative *in vitro* analysis is performed to determine the costimulatory function of poB7-2 or poB7-1 in the context of the human MHC class II molecule HLA-DR1, with that of human B7-1 or B7-2 in the context of DR1, in proliferation assays with human or porcine responders.

(II) Transfected P815 cells are crucial reagents for the detection of porcine anti-B7-2 antibody in the sera of immunised mice which have undergone the chimeric peptide immunisation regimen. Flow cytometric analysis with control or poB7-2 -transfected P815 cells, reflects the specificity of sera for B7-2. Preliminary studies with C57BL-6 mice immunised with a pool of all nine B7-2 peptides have demonstrated the preferential binding of B7-2 peptide sera to porcine B7-2 transfected P815 cells (Figure 11a and 11b).

(III) Mab with specificity for poB7-2 are generated by immunisation of Balb/c mice with poB7-2 expressing P815 cells . The spleens from immunised mice are fused with the NS0 fusion partner and successful fusion's selected by virtue of HAT selection. Flow cytometric staining of poB7-2 P815 transfectants with culture supernatants enable the identification of MAb secreting cells. Cells are grown in culture and the medium harvested for antibody purification by passage over Protein G following ammonium sulphate precipitation. Techniques for the preparation on monoclonal antibodies are well

known in the art and with reference to publications such as Harlow and Lane Antibodies; A Laboratory Manual; Cold Spring Harbour Laboratories.

MAb with specificity for B7-1 and CD40 are generated using the same protocol. These MAb will provide valuable reagents for further characterising the expression of CS molecules on relevant porcine tissues.

5.3 Design and synthesis of poB7-2/OVA chimeric peptide constructs

Nine different peptides derived from the sequence of poB7-2 were initially selected for synthesis. Porcine B7-2 peptides, 6-22mer in size, were selected as determined by the predicted size of a B cell epitope. Peptides were selected for synthesis in combination with a T cell epitope OVA 323-339. B7-2 peptides were selected on the basis of 3D computer modelling (in collaboration with Paul Travers) and on the basis of predicted antigenicity and hydrophilicity using the SeqAid II computer software package. All of the nine peptides reflect linear epitopes. The positions of the nine peptides in the cloned poB7-2 sequence are indicated (Figure 12). Synthetic peptide sequences are detailed in Table 1

Table 1

Peptide Name	Peptide Sequence	Position
Peptide 1	ISQAVHAAHAEINEAGRSFDQATWTLR	81-90
Peptide 2	ISQAVHAAHAEINEAGRLPCHFTNSQ	32-40
Peptide 3	ISQAVHAAHAEINEAGRKGPHGLVPIHQMS	109-121
Peptide 4	ISQAVHAAHAEINEAGRGLVPIHQMS	113-121
Peptide 5	ISQAVHAAHAEINEAGRVQIKDKGSYQC	94-104
Peptide 6	ISQAVHAAHAEINEAGRCSSTQGYPEPQR	151-162
Peptide 8	ISQAVHAAHAEINEAGRKSQAYFNETGEL	21-32
Peptide 9	ISQAVHAAHAEINEAGRASLKSQAYFNET	17-29
Peptide 10	ISQAVHAAHAEINEAGRYMGRTSFDQATWT	76-88
Ova Peptide	ISQAVHAAHAEINEAGR	323-339

The peptide sequences and amino acid positions for peptides 1-10 relate to the position of the B7-2 peptide sequence within porcine B7-2. The amino acid position for the ova sequence is only indicated for the Ova peptide. A 17 amino acid peptide from chicken egg albumin (ovalbumin) was selected as the T cell epitope, OVA323-339 (ISQAVHAAHAEINEAGR). This epitope was selected on the basis of published reports for the generation of a H-2^b restricted T cell response (76,77).We have demonstrated the ability of C57BL-6 mice (H-2^b haplotype) to mount a proliferative response to both the native molecule and to the OVA 323-339 peptide following immunisation with whole ovalbumin (Figure 13). Peptides were generated on a peptide synthesiser (Genosys) and crude peptides were purified by HPLC to greater than 70% purity. Sera from OVA control immunised mice should ideally not recognise the 323-339 sequence, indicating that the T cell epitope is devoid of B cell determinants.

5.4 Tolerance induction

5.4.1 *In vivo* tolerance induction strategy

C57BL-6 mice are immunised with whole ovalbumin in CFA, followed by either control peptide (OVA peptide) or CS peptides (OVA-B7-2 constructs) for three weekly immunisations. Blood is collected following sacrifice and sera prepared using a standard

technique. Presence of specific mouse anti-porcine B7-2 IgG and/or IgM Ab is detected by one of two strategies.

Peptide ELISAs are used to screen for the presence of anti-peptide antibody in the sera. Peptides are coated to plates by virtue of aldehyde linkages to allow free access of Ab to the peptide (78), Plates are coated with individual peptides or the ova control peptide to enable the identification of specific peptides of interest. To detect reactivity of sera with the native B7-2 molecule expressed on the surface of PoB7-2 transfected P815 cells, flow cytometry is performed following surface staining. Having identified CS peptide of interest (peptide ELISA positive and recognising native B7-2) the sera is used to inhibit *in vitro* T cell proliferative responses. This determines whether the antibody is a blocking antibody.

In vivo studies are performed using the islet transplant system. Antibodies which recognise the native molecule but fail to block a proliferative response are useful polyclonal antibody reagents.

Immunisations involved two groups of mice, one received a pool of all nine B7-2 peptides, and one receiving ova control peptide. The harvested sera were screened by peptide ELISA (Figure 14a or 14b) which enabled the identification of peptides of interest. Antisera to peptides 2, 4 and 6 clearly demonstrate preferential binding to B7 peptide than to ova control. The sera has also demonstrated enhanced binding to poB7-2 transfected cells (Figure 11). Peptide 4 and 6 were selected as candidate peptides and used in subsequent immunisation protocol. Immunisation with peptide 4 or 6 clearly produced a significant level of IgG with specificity for peptides 4 and 6 in the sera of immunised mice (Figure 15a and 15b). The specificity of the sera for peptide 4 and not to ova control is demonstrated in Figure 16. The ability of sera from peptide 4 and 6 immunised mice to specifically recognise the native porcine B7-2 molecule expressed on the surface of porcine B7-2 transfected P815 cells is illustrated in Figure 17a and 17b. Untransfected control P815 cells do not stain with the Peptide 4 or 6 sera, neither do

control or transfected cells incubated with ova peptide sera. Similar protocols will be followed with peptide 2. These data clearly demonstrate the ability of this technique to generate anti-peptide antibody directed against an amino acid sequence, by virtue of a carrier T cell epitope.

An identical strategy will be followed with peptides designed on the basis of porcine CD40 and porcine B7-1 once the DNA sequence encoding these molecules has been elucidated.

5.4.2 Functional assessment; prolongation of pancreatic islet xenograft survival

Islet xenografts being non-vascular are rejected solely by T cell mediated mechanisms (79,80), thereby providing an ideal system to study modulation of T cell mediated reactions, please see Figure 18. A very clear role for cell mediated rejection of islets has been demonstrated and is reported to be greater than the comparable alloresponse (80). Transplantation of porcine pancreatic islets to mice is an established procedure, which is well documented in the literature (80-83). Studies within this laboratory have demonstrated a decrease in hyperglycaemia (Figure 18) following transplantation of pancreatic islets from large white pigs under the kidney capsule of C57BL-6 mice rendered diabetic by intraperitoneal administration of streptozotocin, please see Figure 19 and 20. Further optimisation of the isolation procedure (84,85) is required to enable purification of fully functional islets. Transplanted islets usually survive between 6-10 days in the absence of any immunosupression. Successful modulation of direct T cell mediated xenorejection will be monitored by prolongation of islet survival beyond day 10, with comparison to the appropriate controls.

The results obtained with B7-2 to date, demonstrate the ability of synthetic B7-2 peptides conjugated to a known T cell helper epitope to generate the production of anti-pocine B7-2 antibody *in vivo*. These antibodies if directed towards the binding site between B7 isoforms and CD28, in association with antibodies directed against CD40-CD40L will

block the costimulation of human T cells with direct anti-pig xenoreactivity thereby prolonging islet survival in a xenotransplantation context.

Having established the suitability of such an approach in a pig islet to mouse *in vivo* model, studies would progress to pig to primate transplantation systems prior to clinical trials.

5.5 Adaptations for clinical use of these strategies

For clinical applicability the following requirements are necessary:

(I) selection of a suitable T cell epitope to replace OVA. One candidate molecule is tetanus toxiod (TT) which is a widely used antigen for use in human immunisation strategies (68,86). The prior immunisations of most adults with TT is an additional benefit to this strategy as memory T cells are already present in the circulation.

(ii) An efficient and rapid screening method is used to detect the presence of anti-donor (pig) B7-2 antibodies in the absence of a specific B7-2 directed T cell response generated by the recipient which would accelerate graft rejection.

6. SUMMARY OF SPECIFIC EMBODIMENTS

The above examples relate to a novel strategy to inhibit costimulation by porcine cells of human T cells with direct anti-pig xenoreactivity. This is of particular importance in the context of xenotransplantation of porcine organs due to the expression of costimulatory molecules on porcine endothelial, as well as bone marrow-derived antigen presenting cells.

Recipients are immunised with hybrid synthetic peptides comprising a T cell epitope conjugated to sequences of the porcine costimulatory molecules, CD80, CD86 and CD40. Peptides that induce antibodies specific for regions of the costimulatory molecules involved in binding to their counter-receptors on human cells (CD28 and CD154) are therefore capable of blocking the delivery of costimulation. Once the antibody response has been induced, the transplanted organ will recall this response due to the expression of

the costimulatory molecules, thereby sustaining this response, and providing an endogenous mechanism of costimulatory blockade.

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CD86 (B7-2)

Human and porcine CD86 protein sequences were aligned and regions of non-homology identified. We predict that the peptide sequences will be derived from those regions listed below or from any overlap regions between any of these peptides.
The sequences of predicted interest for containing potential antibody epitopes have been selected on the basis of less than 75% sequence identity.

Region	Position	% sequence identity
i	18-42	72%
ii	55-73	55%
iii	101-127	63%
iv	136-165	56%

Regions (iii) and (iv) encompass those containing the peptide 4 and 6 sequences identitifed in mice.

CD40

Human and porcine CD40 protein sequences were aligned and regions of non-homology identified. We predict that the peptide sequences will be derived from those regions listed below or from any overlap regions between any of these peptides.
The sequences of predicted interest for containing potential antibody epitopes have been selected on the basis of less than 75% sequence identity.

Region	Position	% sequence identity
i	25-48	63%
ii	49-75	74%
iii	93-114	59%
iv	123-139	63%
v	158-176	68%
vi	208-227	45%
vii	231-248	21%

VCAM-1

Human and porcine VCAM-1 protein sequences were aligned and regions of non-homology identified. We predict that the peptide sequences will be derived from those regions listed below or from any overlap regions between any of these peptides.
The sequences of predicted interest for containing potential antibody epitopes have been selected on the basis of less than 75% sequence identity.

Region	Position	% sequence identity
i	1-15	44%
ii	16-33	63%
iii	49-65	58%
iv	74-85	42%
v	100-117	50%
vi	122-140	56%
vii	144-157	64%
viii	162-191	47%
ix	209-221	62%
x	290-301	67%
xi	322-342	62%
xii	362-379	67%
xiii	448-465	67%

CLAIMS

1. A method of improving tolerance to a porcine xenograft comprising immunising a mammal with an immunogen comprising:

i) a T- cell epitope; and

ii) a B-cell epitope characterised in that the B-cell epitope is a porcine polypeptide involved in mediating xenograft rejection and derived from a region of a porcine polypeptide which has less than 75% sequence identity to the corresponding region of the equivalent human polypeptide.

2. A method according to Claim 1 wherein the B-cell epitope is a peptide derived from at least one porcine polypeptide selected from; CD40; CD80; CD86 or VCAM.

3. A method according to Claim 1 or 2 wherein the peptide is selected from at least one peptide represented in Figure 22.

4. A method according to Claim 1 or 2 wherein the peptide is selected from at least one peptide represented in Figure 24.

5. A method according to Claim 1 or 2 wherein the peptide is selected from at least one peptide represented in Figure 26.

6. A method according to any of Claims 1-5 wherein the T – cell epitope is derived from tetanus toxoid polypeptide.

7. A composition comprising an immunogen characterised in that the immunogen has a T – cell epitope and a B- cell epitope wherein the B – cell epitope is derived from a region of a porcine polypeptide which has less than 75% sequence identity to the corresponding region of the equivalent human polypeptide.

8. A composition according to Claim 7 wherein the porcine polypeptide is expressed by vascular endothelial cells of said xenograft.

9. A composition according to Claims 7 or 8 wherein the B–cell epitope is derived from at least one porcine polypeptide selected from; CD40; CD86; CD80; VCAM.

10. A composition according to Claim 9 wherein the B- cell epitope is selected from at least one peptide as represented in Figure 22 .

11. A composition according to Claim 9 wherein the B- cell epitope is selected from at least one peptide as represented in Figure 24 .

12. A composition according to Claim 9 wherein the B- cell epitope is selected from at least one peptide as represented in Figure 26.

13. A compostion according to Claims 9 or 12 wherein the B- cell epitope is derived from the extracellular domain of CD86.

14. A composition according to any of Claims 7 - 13 wherein the T- cell epitope is derived from tetanus toxoid.

15. A composition according to any of Claims 7 - 14 wherein the composition further comprises a carrier capable of enhancing the immune response to said immunogen.

16. An antibody, or the effective part thereof, characterised in that said antibody is capable of binding to a region of a porcine polypeptide which has less than 75% sequence identity to the corresponding region of the equivalent human polypeptide.

17. An antibody according to Claim 16 wherein the antibody is a monoclonal antibody.

18. An antibody according to Claims 16 or 17 wherein the antibody is modified with at least one detectable label.

19. A method to monitor the immune status of a mammalian recipient of a xenograft comprising:

i) removing a sample from a xenograft recipient to be tested;

ii) contacting said sample with the antibody according to Claims 16 -18; and

iii) monitoring the expression of a porcine polypeptide involved in mediating xenograft rejection.

20. A method to treat a mammal prior to receiving a xenograft comprising:

i) immunising a mammal with a composition according to Claims 7-15;

ii) assessing the immune status of said mammal to said immunogenic composition;

iii) transplantation of said xenograft tissue/organ into a recipient mammal; and

iv) monitoring the rejection response to said xenograft.

21. A method according to Claim 19 or 20 wherein the xenograft is of porcine origin and said mammal is human.

22. A method according to any of Claims 19 -21 wherein the xenograft is at least one vascularised graft and/or immunogenic porcine cell/tissue.

23. A method according to any of Claims 19 – 22 wherein the xenograft is pancreatic islets.

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(57) Abstract

The invention hereindescribed relates to a method to improve the tolerance of a mammal, preferably a human, to a xenograft through immunisation of the recipient mammal with an immunogen comprising both a B cell epitope derived from porcine polypeptides and T cell epitope. The invention also encompasses immunogenic compositions comprising said immunogens and methods to monitor the status of the xenograft.

Figure 1

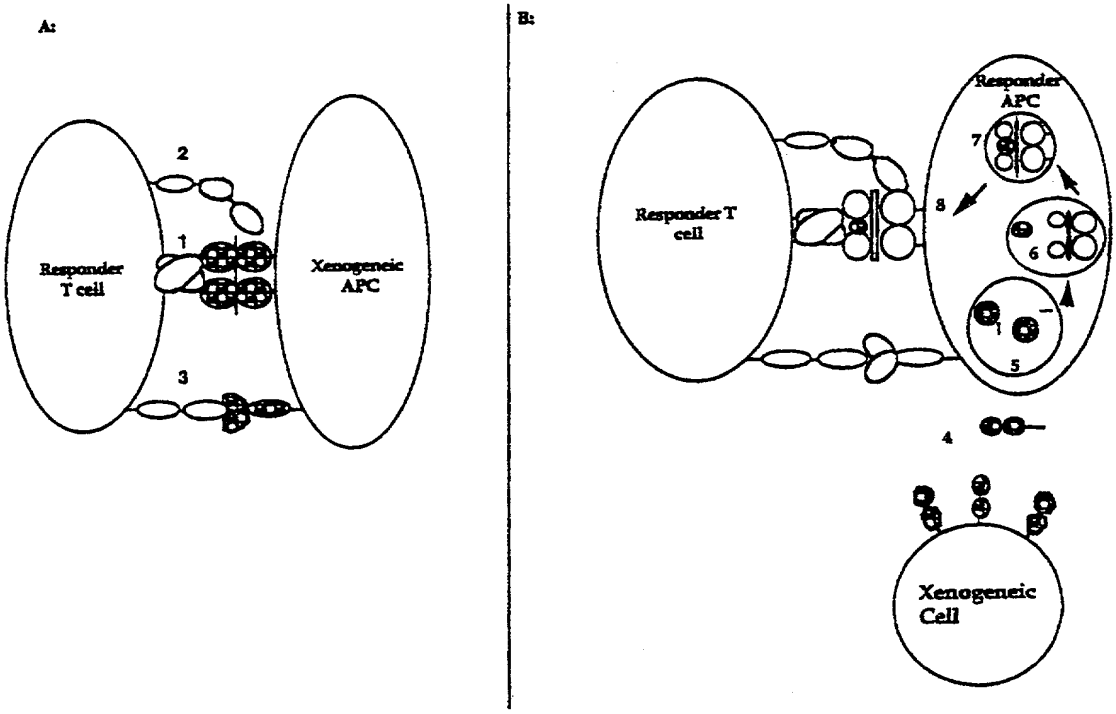

A:
Responder T cell
Xenogeneic APC
1
2
3
B:
Responder T cell
Responder APC
7
8
6
5
4
Xenogeneic Cell

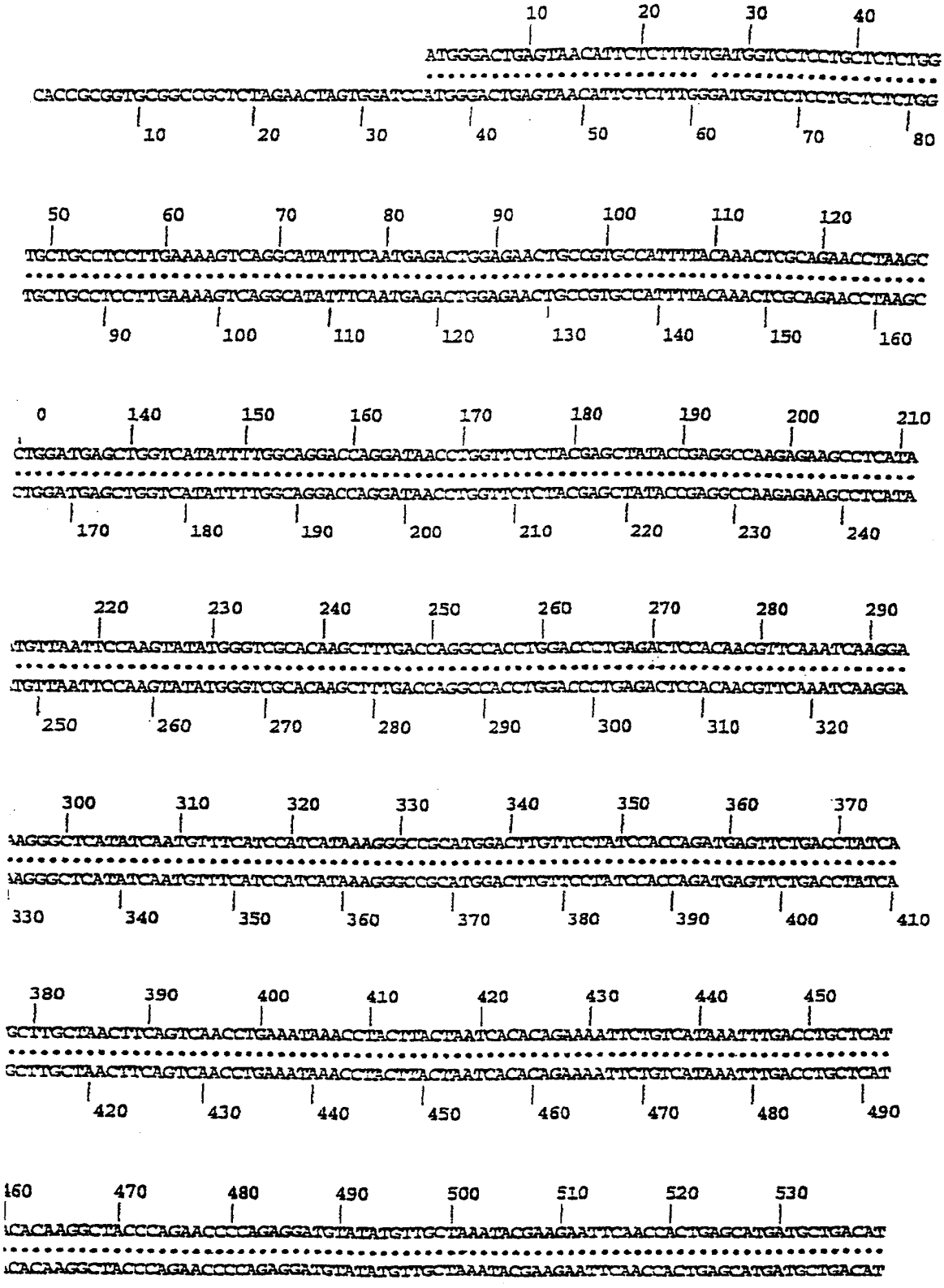
Figure 2

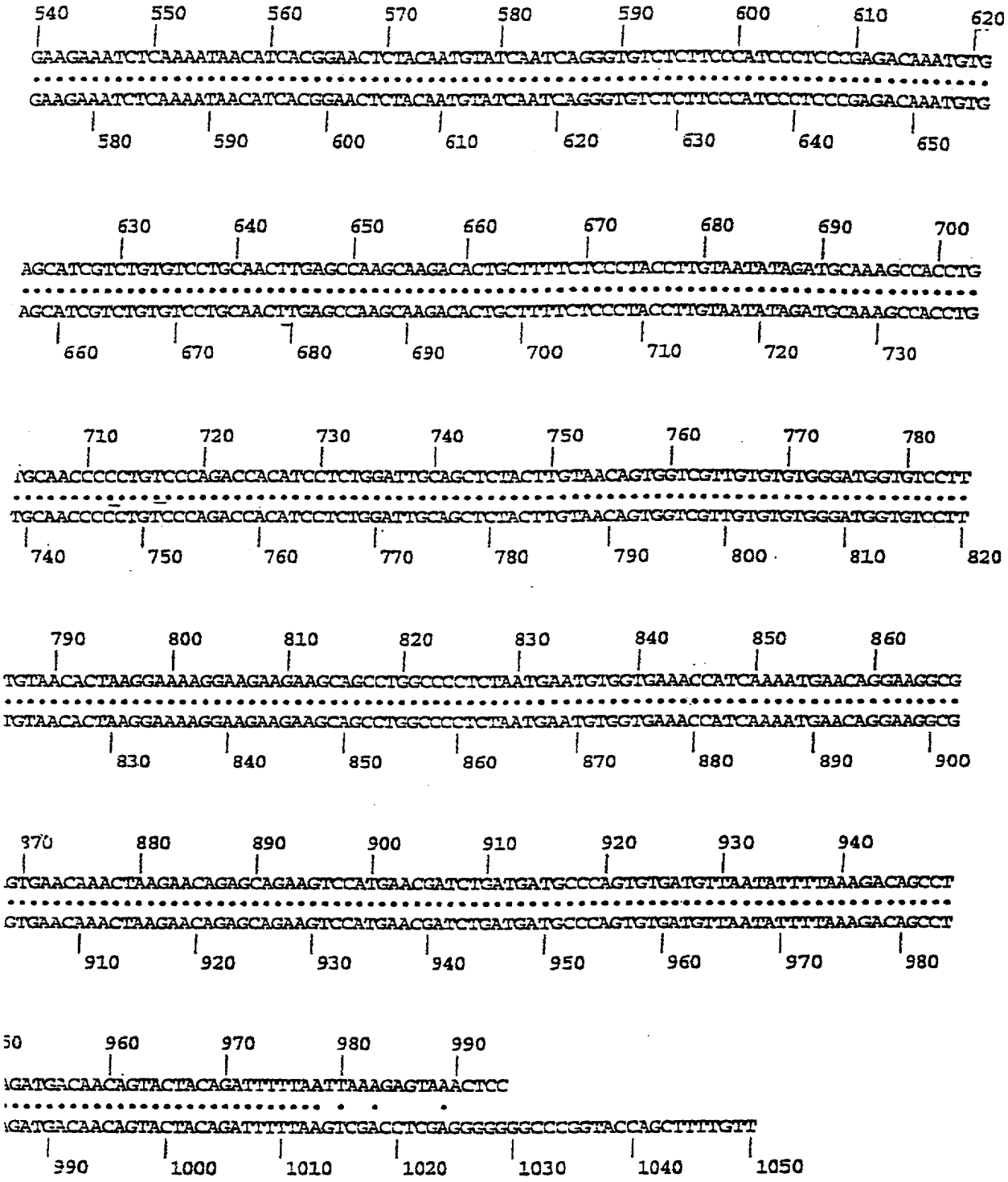
```
        GCATGGATCCATGGGACTGAGTAACATTCTCTTTG
1                ATGGGACTGAGTAACATTCTCTTTGTGATGGTCCTCCT
39      GCTCTCTGGTGCTGCCTCCTTGAAAAGTCAGGCATATTTCAATGAGA
86      CTGGAGAACTGCCGTGCCATTTTACAAACTCGCAGAACCTAAGCCTG
133     GATGAGCTGGTCATATTTTGGCAGGACCAGGATAACCTGGTTCTCTA
181     CGAGCTATACCGAGGCCAAGAGAAGCCTCATAATGTTAATTCCAAG
227     TATATGGGTCGCACAAGCTTTGACCAGGCCACCTGGACCCTGAGACT
274     CCACAACGTTCAAATCAAGGACAAGGGCTCATATCAATGTTTCATC
321     CATCATAAAGGGCCGCATGGACTTGTTCCTATCCACCAGATGAGTTC
368     TGACCTATCATTGCTTGCTAACTTCAGTCAACCTGAAATAAACCTAC
415     TTACTAATCACACAGAAAATTCTGTCATAAATTTGACCTGCTCATCT
462     ACACAAGGCTACCCAGAACCCCAGAGGATGTATATGTTGCTAAATA
509     CGAAGAATTCAACCACTGAGCATGATGCTGACATGAAGAAATCTCA
556     AAATAACATCACGGAACTCTACAATGTATCAATCAGGGTGTCTCTT
602     CCCATCCCTCCCGAGACAAATGTGAGCATCGTCTGTGTCCTGCAACTT
649     GAGCCAAGCAAGACACTGCTTTTCTCCCTACCTTGTAATATAGATGC
696     AAAGCCACCTGTGCAACCCCCTGTCCCAGACCACATCCTCTGGATTGC
743     AGCTCTACTTGTAACAGTGGTCGTTGTGTGTGGGATGGTGTCCTTTGT
790     AACACTAAGGAAAAGGAAGAAGAAGCAGCCTGGCCCCTCTAATGA
837     ATGTGGTGAAACCATCAAAATGAACAGGAAGGCGAGTGAACAAAC
884     TAAGAACAGAGCAGAAGTCCATGAACGATCTGATGATGCCCAGTGT
931     GATGTTAATATTTTAAAGACAGCCTCAGATGACAACAGTACTACAG
                                        GACAACAGTACTACAG
978     ATTTTTAATTAAAGAGTAAACTCC
        ATTTTTAAGTCGACATGC
```

FIGURE 3

```
   1  CACCGCGGTG CGGCCGCTCT AGAACTAGTG GATCCATGGG ACTGAGTAAC
  51  ATTCTCTTTG GGATGGTCCT CCTGCTCTCT GGTGCTGCCT CCTTGAAAAG
 101  TCAGGCATAT TTCAATGAGA CTGGAGAACT GCCGTGCCAT TTTACAAACT
 151  CGCAGAACCT AAGCCTGGAT GAGCTGGTCA TATTTTGGCA GGACCAGGAT
 201  AACCTGGTTC TCTACGAGCT ATACCGAGGC CAAGAGAAGC CTCATAATGT
 251  TAATTCCAAG TATATGGGTC GCACAAGCTT TGACCAGGCC ACCTGGACCC
 301  TGAGACTCCA CAACGTTCAA ATCAAGGACA AGGGCTCATA TCAATGTTTC
 351  ATCCATCATA AAGGGCCGCA TGGACTTGTT CCTATCCACC AGATGAGTTC
 401  TGACCTATCA GTGCTTGCTA ACTTCAGTCA ACCTGAAATA AACCTACTTA
 451  CTAATCACAC AGAAAATTCT GTCATAAATT TGACCTGCTC ATCTACACAA
 501  GGCTACCCAG AACCCCAGAG GATGTATATG TTGCTAAATA CGAAGAATTC
 551  AACCACTGAG CATGATGCTG ACATGAAGAA ATCTCAAAAT AACATCACGG
 601  AACTCTACAA TGTATCAATC AGGGTGTCTC TTCCCATCCC TCCCGAGACA
 651  AATGTGAGCA TCGTCTGTGT CCTGCAACTT GAGCCAAGCA AGACACTGCT
 701  TTTCTCCCTA CCTTGTAATA TAGATGCAAA GCCACCTGTG CAACCCCCTG
 751  TCCCAGACCA CATCCTCTGG ATTGCAGCTC TACTTGTAAC AGTGGTCGTT
 801  GTGTGTGGGA TGGTGTCCTT TGTAACACTA AGGAAAAGGA AGAAGAAGCA
 851  GCCTGGCCCC TCTAATGAAT GTGGTGAAAC CATCAAAATG AACAGGAAGG
 901  CGAGTGAACA AACTAAGAAC AGAGCAGAAG TCCATGAACG ATCTGATGAT
 951  GCCCAGTGTG ATGTTAATAT TTTAAAGACA GCCTCAGATG ACAACAGTAC
1001  TACAGATTTT TAAGTCGACC TCGAGGGGGG GCCCGGTACC AGCTTTTGTT
```

Figure 4: Comparison of the nucleotide sequence of CD86(i) with the published sequence for porcine CD86.

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580 590 600 610 620 630 640 650
630 640 650 660 670 680 690 700
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710 720 730 740 750 760 770 780
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740 750 760 770 780 790 800 810 820
790 800 810 820 830 840 850 860
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870 880 890 900 910 920 930 940
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50 960 970 980 990
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FIGURE 5

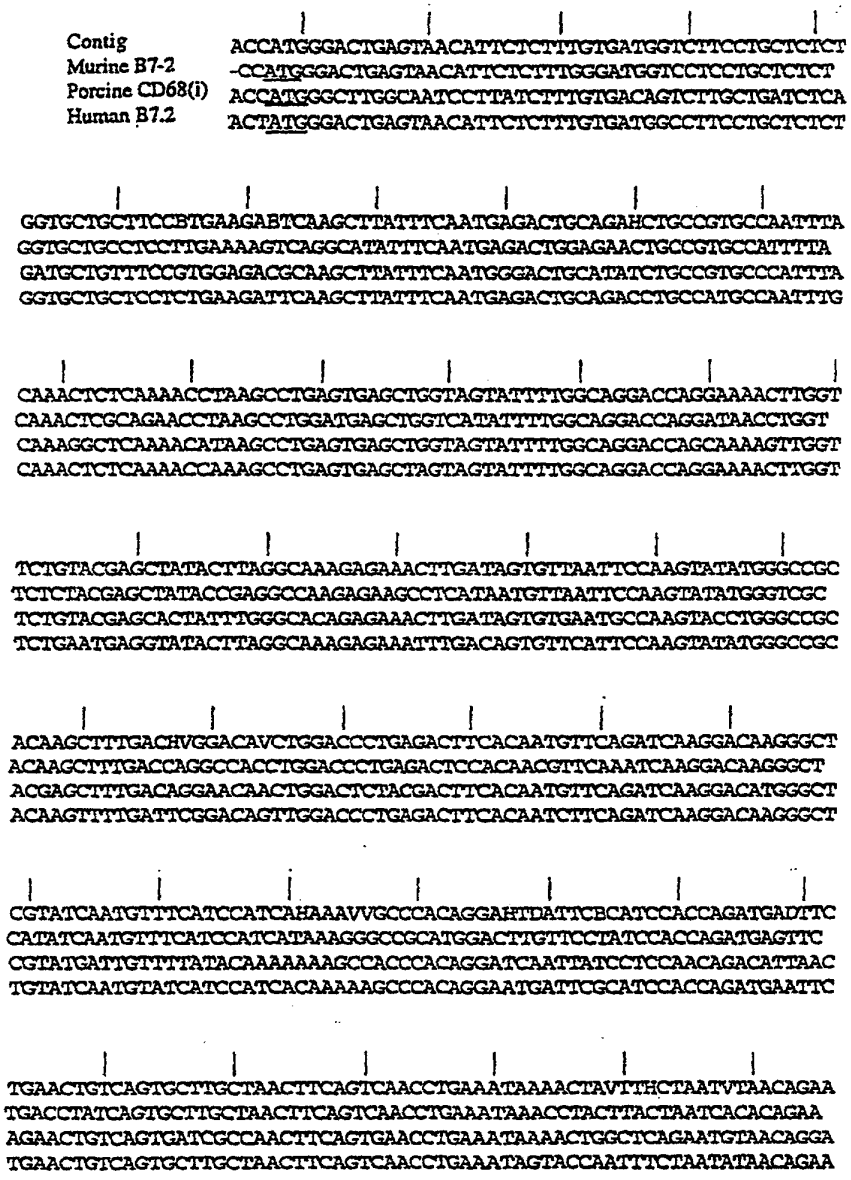
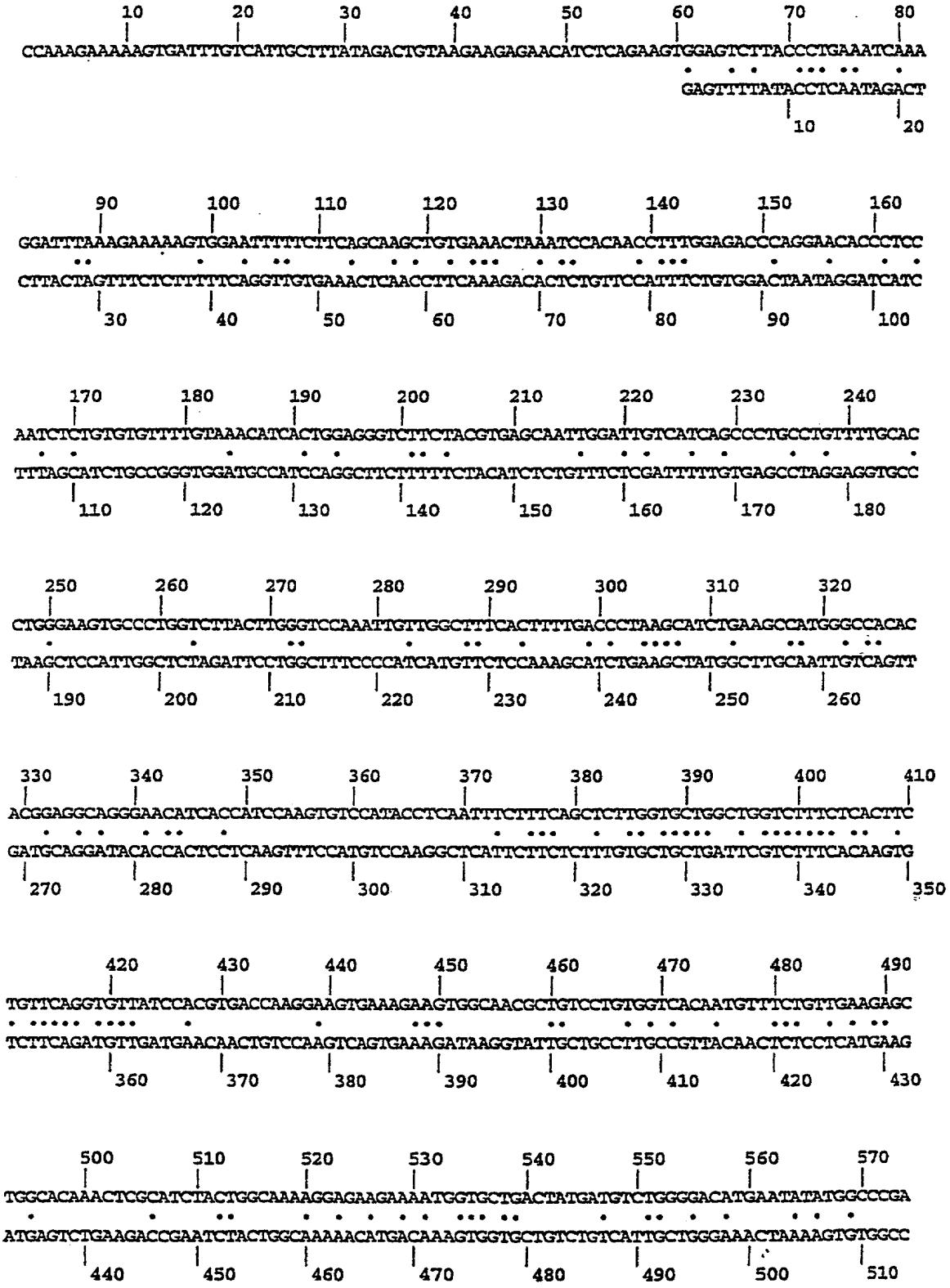


FIGURE 7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10 20
90 100 110 120 130 140 150 160
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170 180 190 200 210 220 230 240
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250 260 270 280 290 300 310 320
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190 200 210 220 230 240 250 260
330 340 350 360 370 380 390 400 410
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420 430 440 450 460 470 480 490
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500 510 520 530 540 550 560 570
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FIGURE 5-1

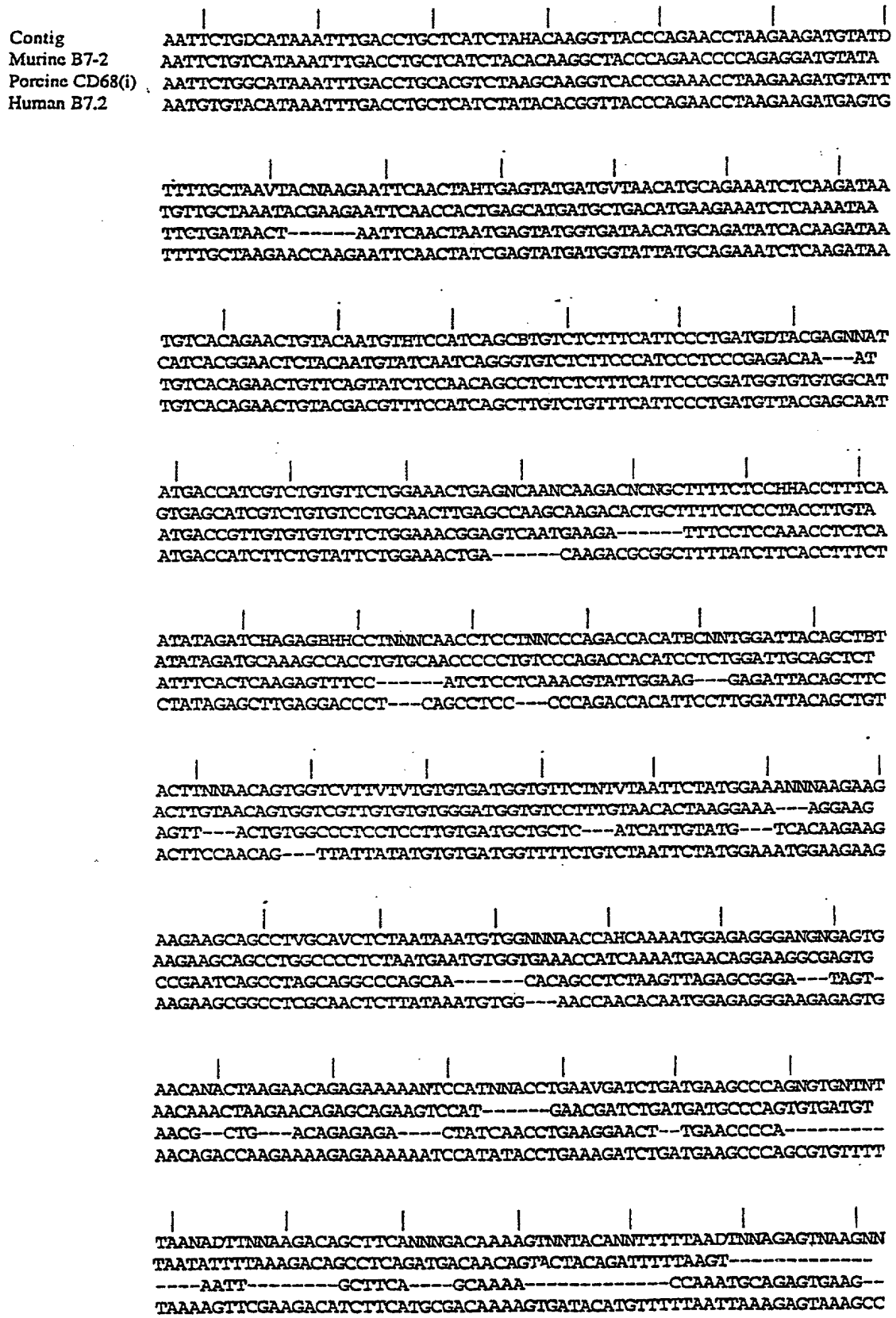


FIGURE 6

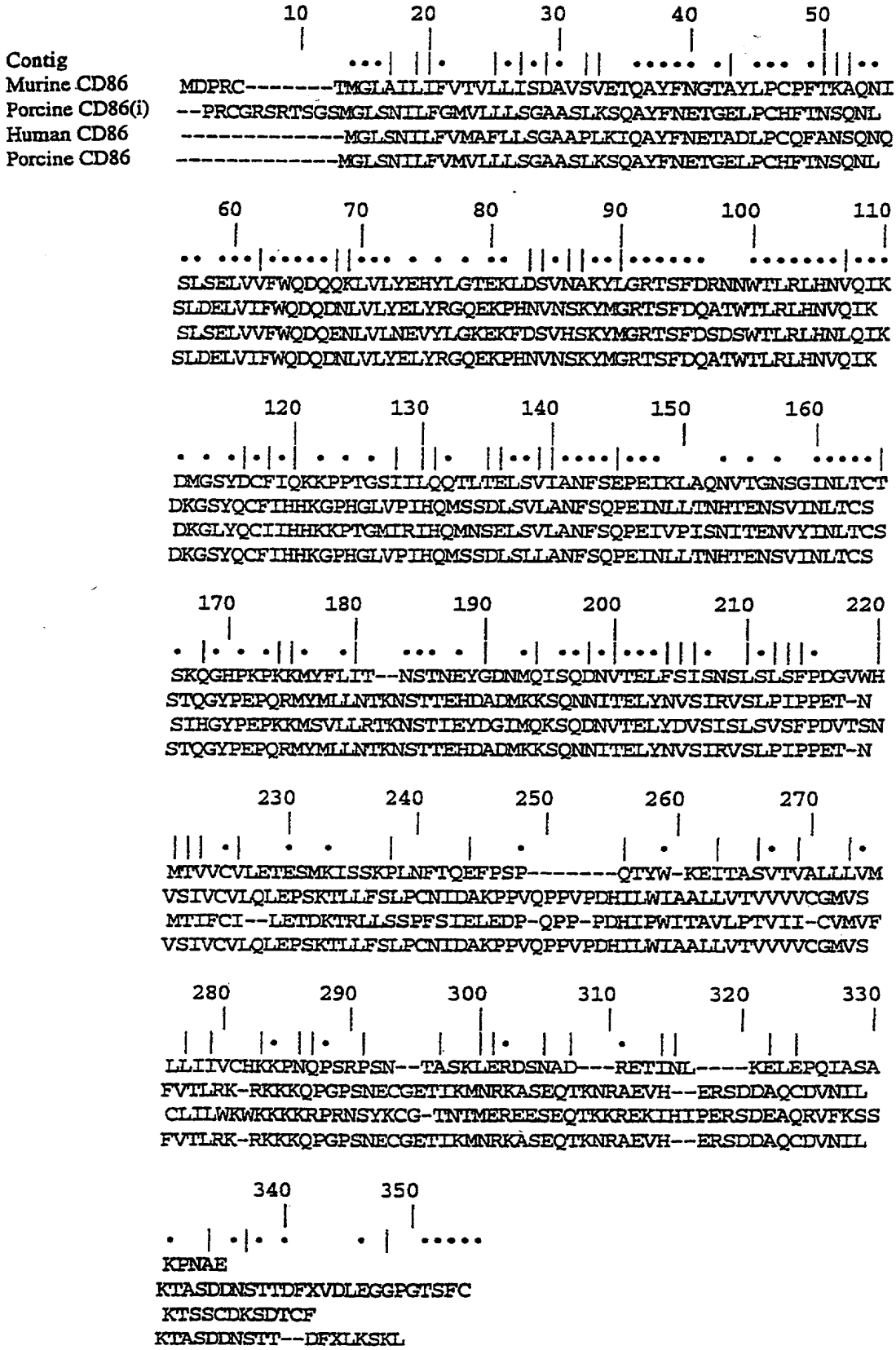
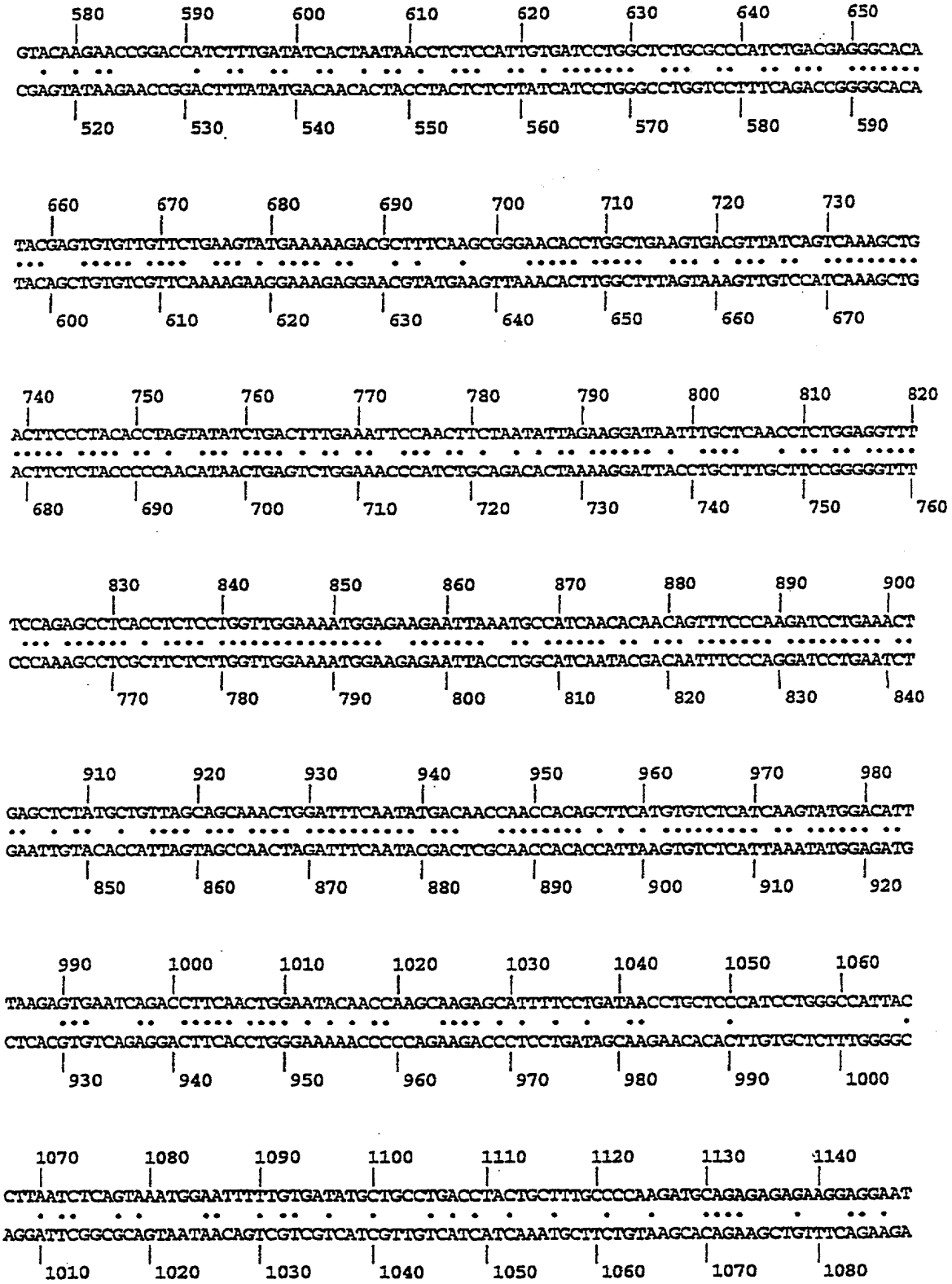


FIGURE 7-1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660 670 680 690 700 710 720 730
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600 610 620 630 640 650 660 670
740 750 760 770 780 790 800 810 820
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830 840 850 860 870 880 890 900
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910 920 930 940 950 960 970 980
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990 1000 1010 1020 1030 1040 1050 1060
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1070 1080 1090 1100 1110 1120 1130 1140
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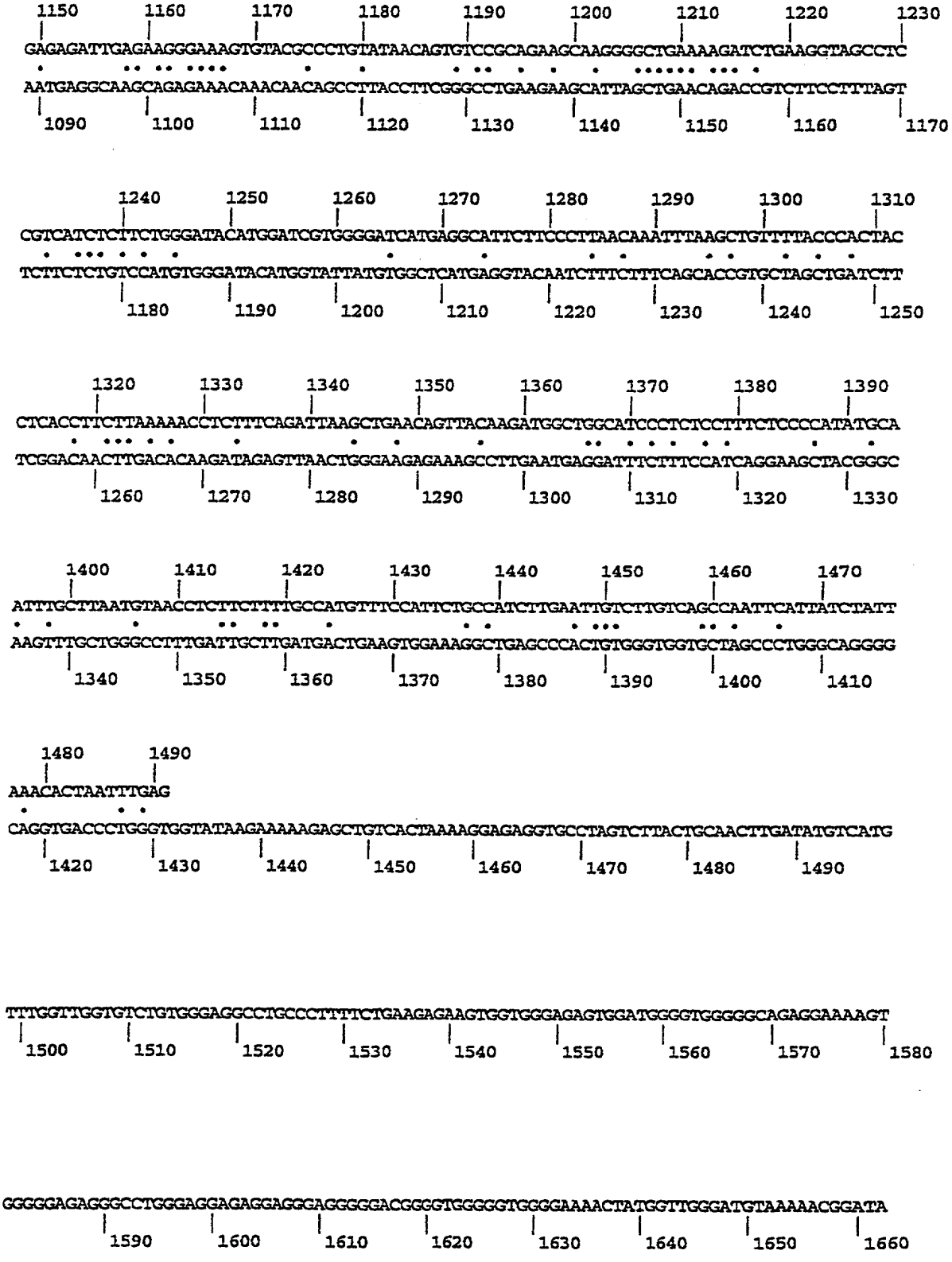
FIGURE 7-2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1480 1490
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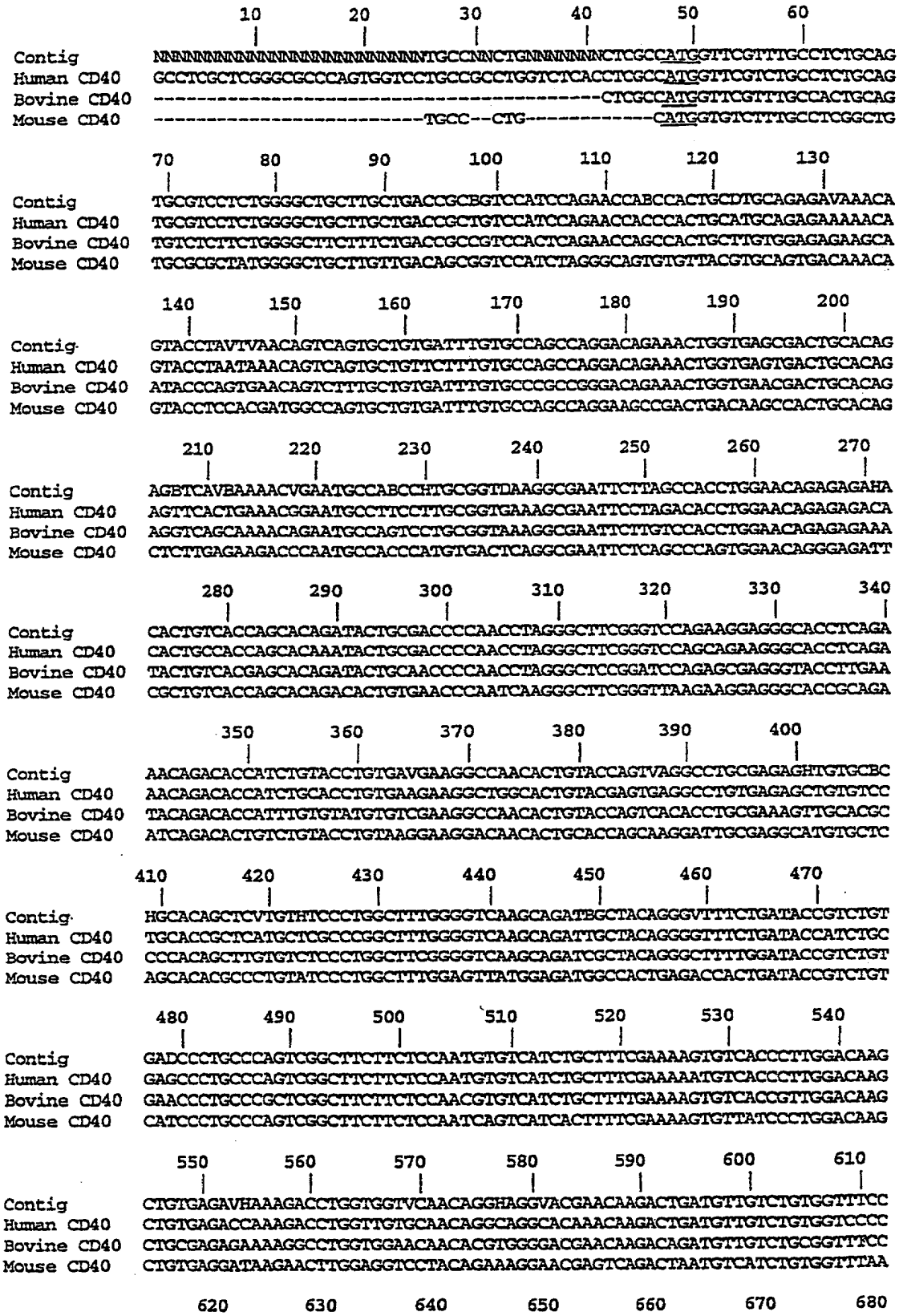
FIGURE 8a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Mouse CD40 ----------TGCC--CTG----------CATGGTGTCTTTGCCTCGGCTG
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Bovine CD40 AGGTCAGCAAAACAGAATGCCAGTCCTGCGGTAAAGGCGAATTCTTGTCCACCTGGAACAGAGAGAAA
Mouse CD40 CTCTTGAGAAGACCCAATGCCACCCATGTGACTCAGGCGAATTCTCAGCCCAGTGGAACAGGGAGATT
280 290 300 310 320 330 340
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Mouse CD40 CGCTGTCACCAGCACAGACACTGTGAACCCAATCAAGGGCTTCGGGTTAAGAAGGAGGGCACCGCAGA
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Bovine CD40 TACAGACACCATTTGTGTATGTGTCGAAGGCCAACACTGTACCAGTCACACCTGCGAAAGTTGCACGC
Mouse CD40 ATCAGACACTGTCTGTACCTGTAAGGAAGGACAACACTGCACCAGCAAGGATTGCGAGGCATGTGCTC
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Bovine CD40 CCCACAGCTTGTGTCTCCCTGGCTTCGGGGTCAAGCAGATCGCTACAGGGCTTTTGGATACCGTCTGT
Mouse CD40 AGCACACGCCCTGTATCCCTGGCTTTGGAGTTATGGAGATGGCCACTGAGACCACTGATACCGTCTGT
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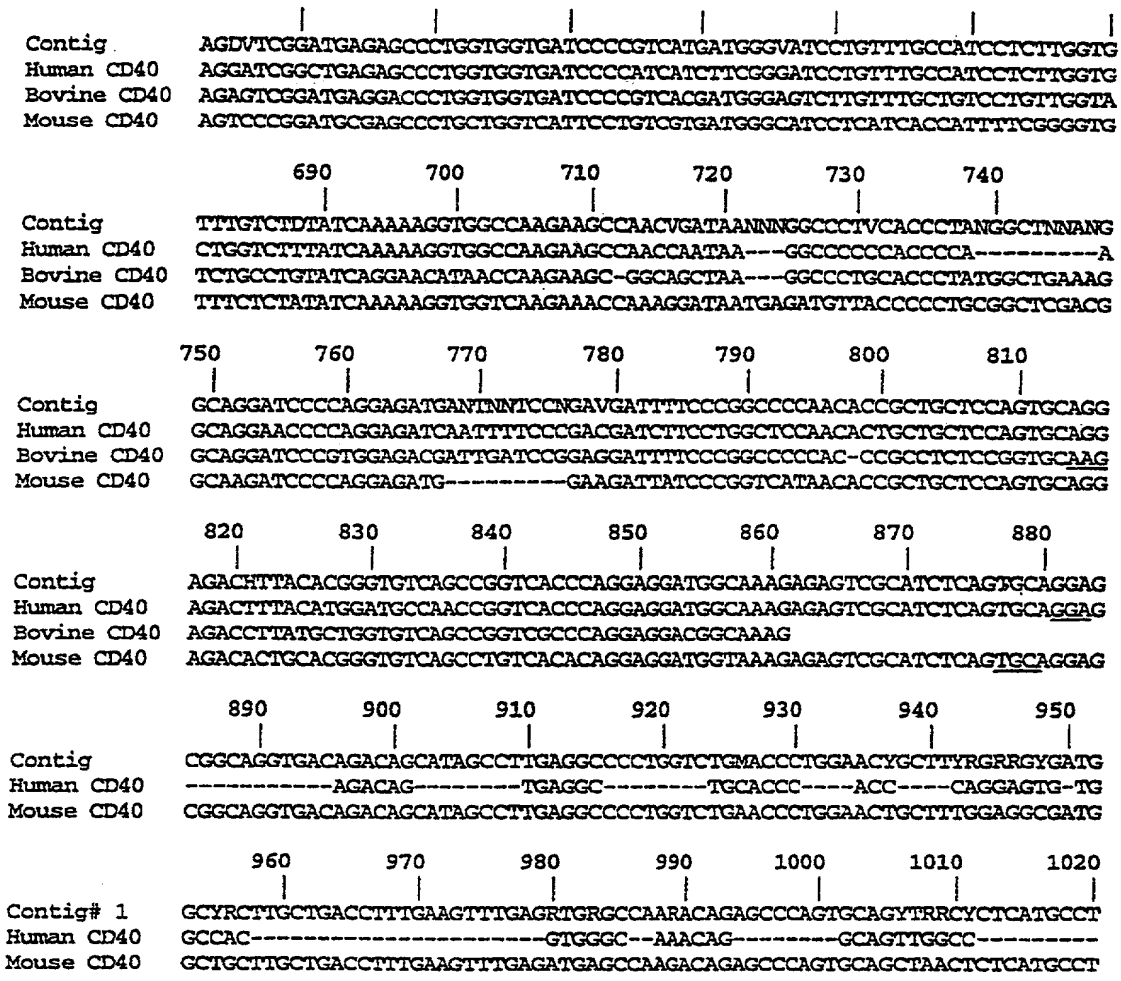
FIGURE 8a - 1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Mouse CD40 AGTCCCGGATGCGAGCCCTGCTGGTCATTCCTGTCGTGATGGGCATCCTCATCACCATTTTCGGGGTG
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Bovine CD40 TCTGCCTGTATCAGGAACATAACCAAGAAGC-GGCAGCTAA---GGCCCTGCACCCTATGGCTGAAAG
Mouse CD40 TTTCTCTATATCAAAAAGGTGGTCAAGAAACCAAAGGATAATGAGATGTTACCCCCTGCGGCTCGACG
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Bovine CD40 GCAGGATCCCGTGGAGACGATTGATCCGGAGGATTTTCCCGGCCCCCAC-CCGCCTCTCCGGTGCAAG
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Bovine CD40 AGACCTTATGCTGGTGTCAGCCGGTCGCCCAGGAGGACGGCAAAG
Mouse CD40 AGACACTGCACGGGTGTCAGCCTGTCACACAGGAGGATGGTAAAGAGAGTCGCATCTCAGTGCAGGAG
890 900 910 920 930 940 950
Contig CGGCAGGTGACAGACAGCATAGCCTTGAGGCCCCTGGTCTGMACCCTGGAACYGCTTYRGRRGYGATG
Human CD40 ----------AGACAG---------TGAGGC---------TGCACCC----ACC-----CAGGAGTG-TG
Mouse CD40 CGGCAGGTGACAGACAGCATAGCCTTGAGGCCCCTGGTCTGAACCCTGGAACTGCTTTGGAGGCGATG
960 970 980 990 1000 1010 1020
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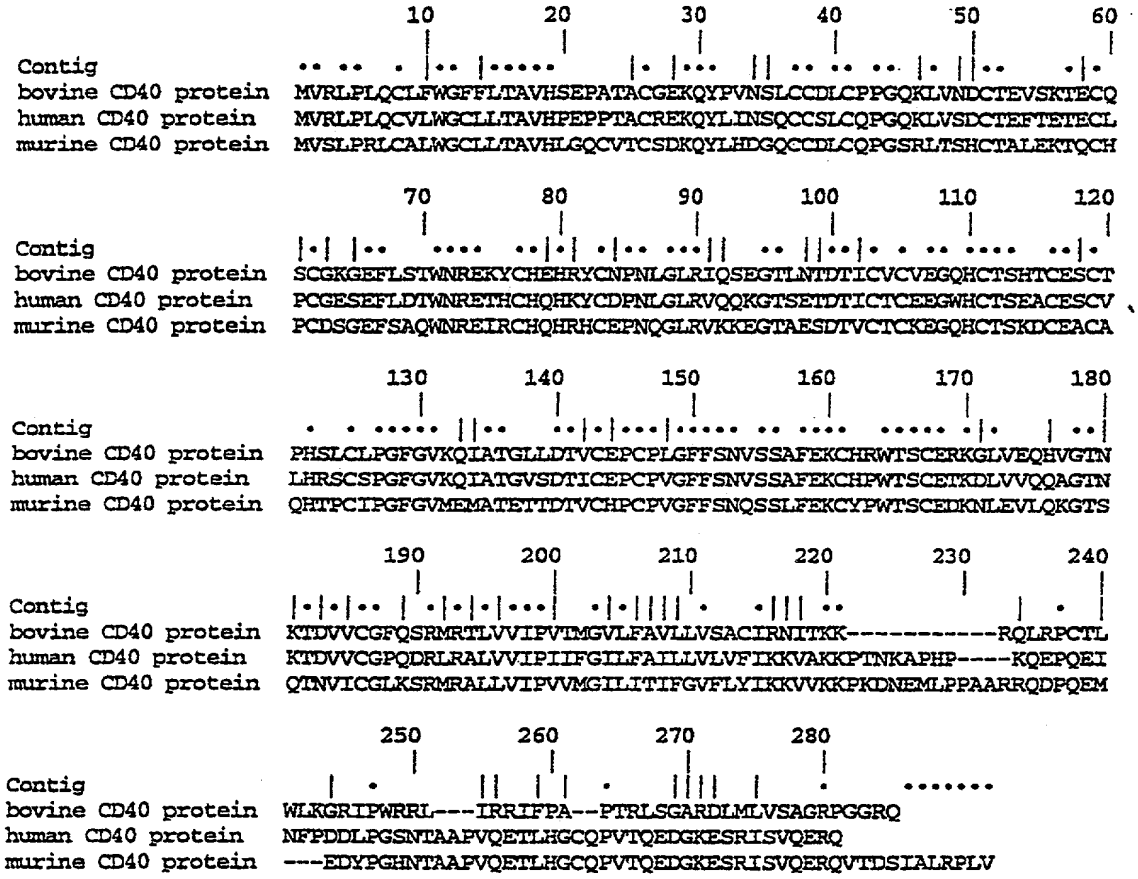
Figure 8b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murine CD40 protein MVSLPRLCALWGCLLTAVHLGQCVTCSDKQYLHDGQCCDLCQPGSRLTSHCTALEKTQCH
70 80 90 100 110 120
Contig
bovine CD40 protein SCGKGEFLSTWNREKYCHEHRYCNPNLGLRIQSEGTLNTDTICVCVEGQHCTSHTCESCT
human CD40 protein PCGESEFLDTWNRETHCHQHKYCDPNLGLRVQQKGTSETDTICTCEEGWHCTSEACESCV
murine CD40 protein PCDSGEFSAQWNREIRCHQHRHCEPNQGLRVKKEGTAESDTVCTCKEGQHCTSKDCEACA
130 140 150 160 170 180
Contig
bovine CD40 protein PHSLCLPGFGVKQIATGLLDTVCEPCPLGFFSNVSSAFEKCHRWTSCERKGLVEQHVGTN
human CD40 protein LHRSCSPGFGVKQIATGVSDTICEPCPVGFFSNVSSAFEKCHPWTSCETKDLVVQQAGTN
murine CD40 protein QHTPCIPGFGVMEMATETTDTVCHPCPVGFFSNQSSLFEKCYPWTSCEDKNLEVLQKGTS
190 200 210 220 230 240
Contig
bovine CD40 protein KTDVVCGFQSRMRTLVVIPVIMGVLFAVLLVSACIRNITKK----------RQLRPCTL
human CD40 protein KTDVVCGPQDRLRALVVIPIIFGILFAILLVLVFIKKVAKKPTNKAPHP----KQEPQEI
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250 260 270 280
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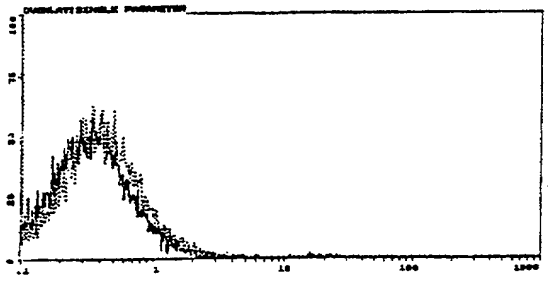
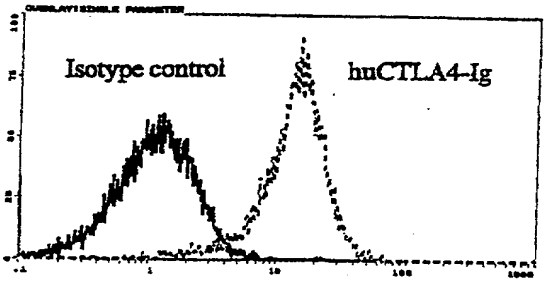
FIGURE 9

A

Non-transfected control cells

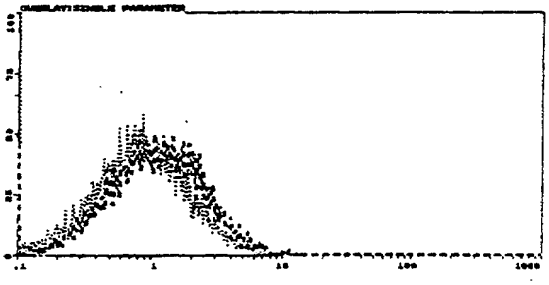


Transfected cells



B

Non-transfected control cells



Transfected cells

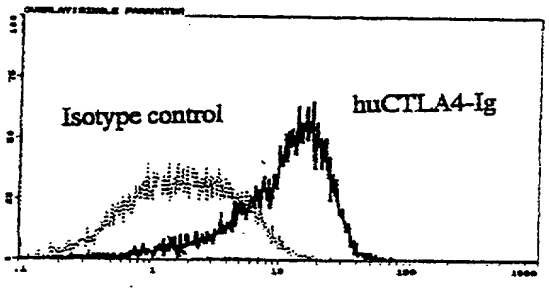
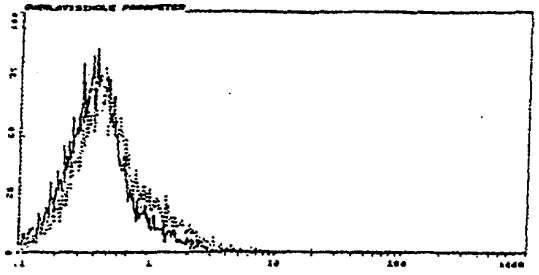
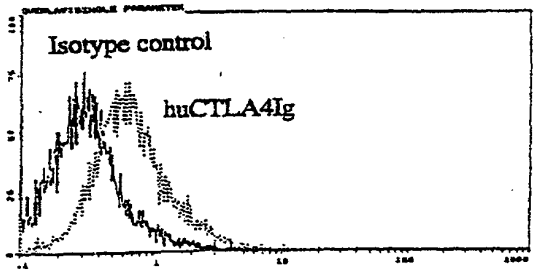


FIGURE 10

Non-transfected control cells

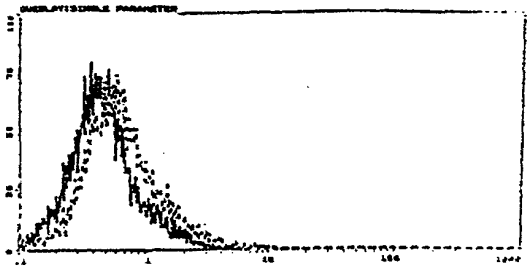
Transfected cells





Non-transfected control cells

Transfected cells



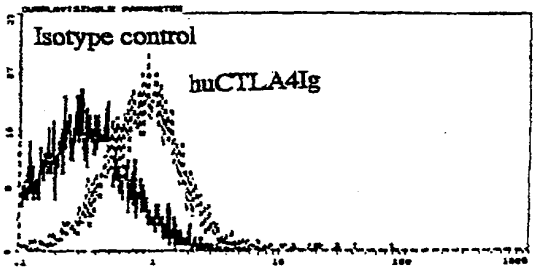
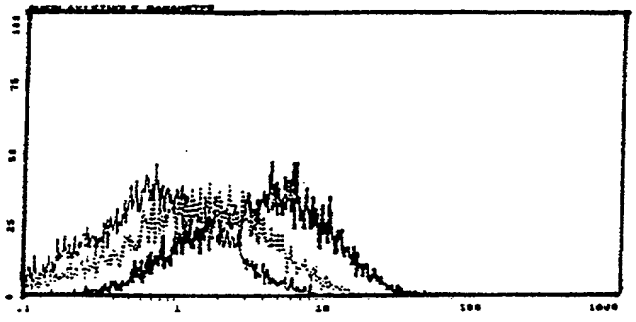


FIGURE 11

A



B

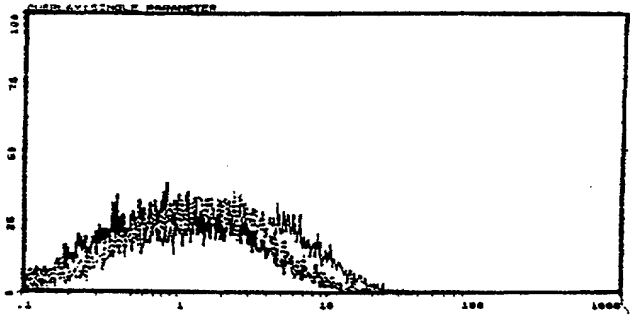


FIGURE 12

1 MGLSNILFVM VLLLSGAASL KSQAYFNETG ELPCHFTNSQ

41 NLSLDELVIF WQDQDNLVLY ELYRGQEKPH NVNSKYMGRT

81 SFDQATWTLR LHNVQIKDKG SYQCFIHHKG PHGLVPIHQM

121 SSDLSLLANF SQPEINLLTN HTENSVINLT CSSTQGYPEP

161 QRMYMLLNTK NSTTEHDADM KKSQNNITEL YNVSIRVSLP

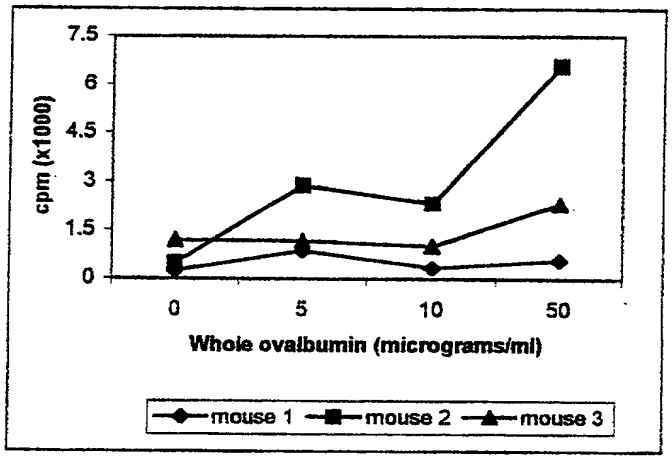
201 IPPETNVSIV CVLQLEPSKT LLFSLPCNID AKPPVQPPVP

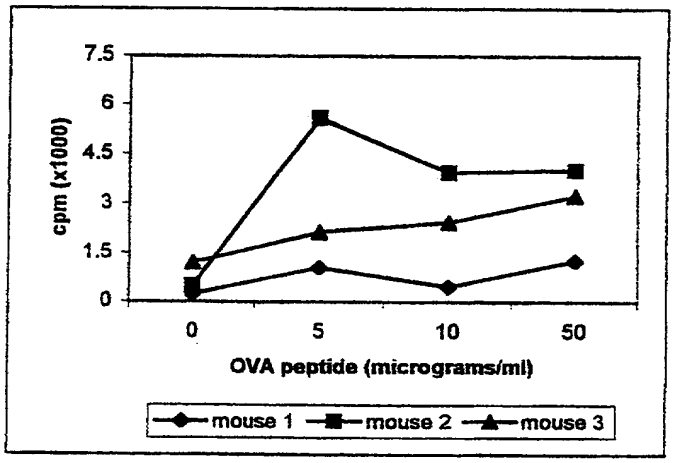
241 DHILWIAALL VTVVVVCGMV SFVTLRKRKK KQPGPSNECG

281 ETIKMNRKAS EQTKNRAEVH ERSDDAQCDV NILKTASDDN

321 STTDF•LKSK L

FIGURE 13


7.5
6
4.5
3
1.5
0
cpm (x1000)
0
5
10
50
Whole ovalbumin (micrograms/ml)
mouse 1
mouse 2
mouse 3


7.5
6
4.5
3
1.5
0
cpm (x1000)
0
5
10
50
OVA peptide (micrograms/ml)
mouse 1
mouse 2
mouse 3

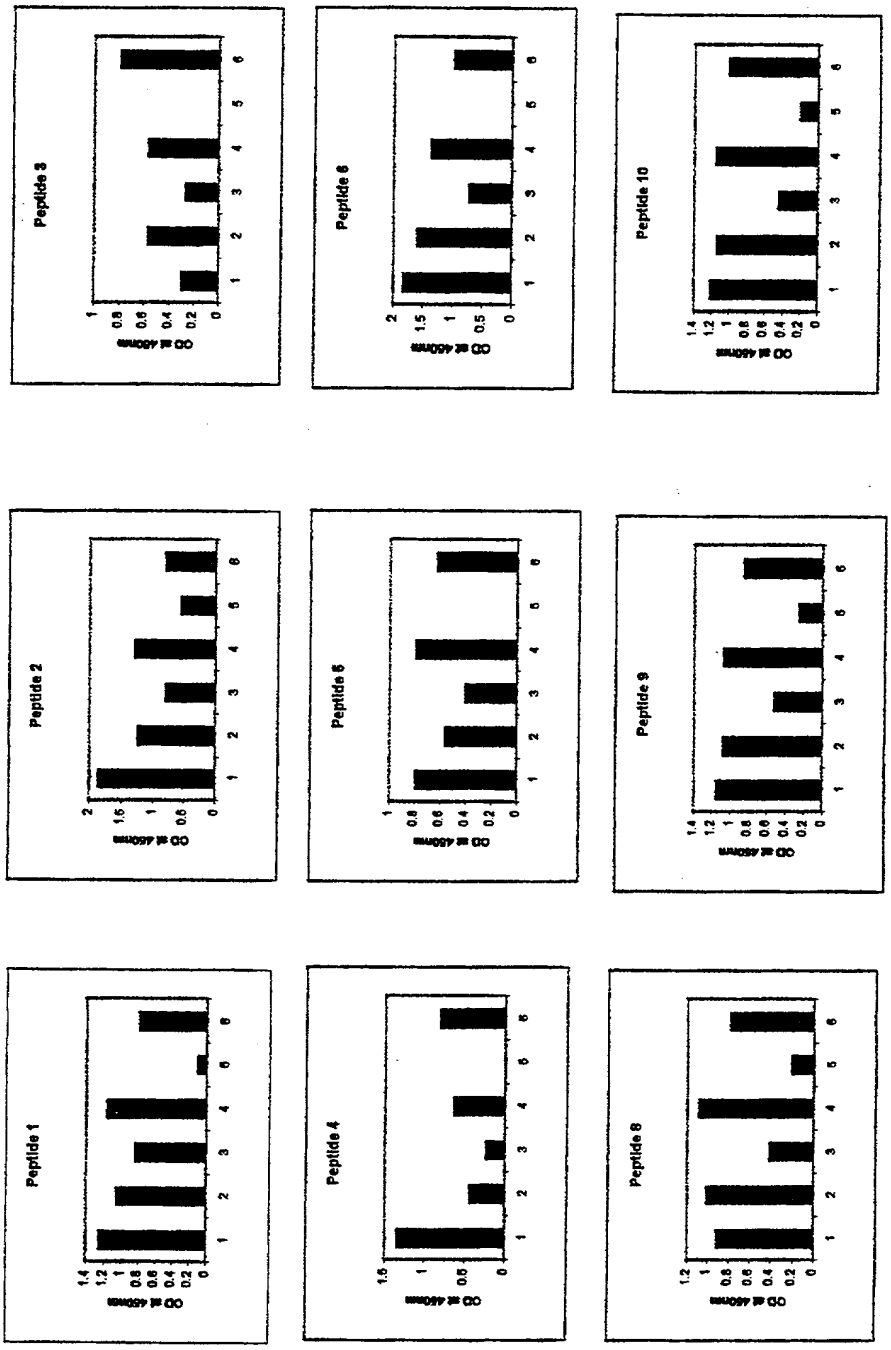

Peptide 3
Peptide 6
Peptide 10
Peptide 2
Peptide 5
Peptide 9
Peptide 1
Peptide 4
Peptide 8

Figure 14a

FIGURE 14b

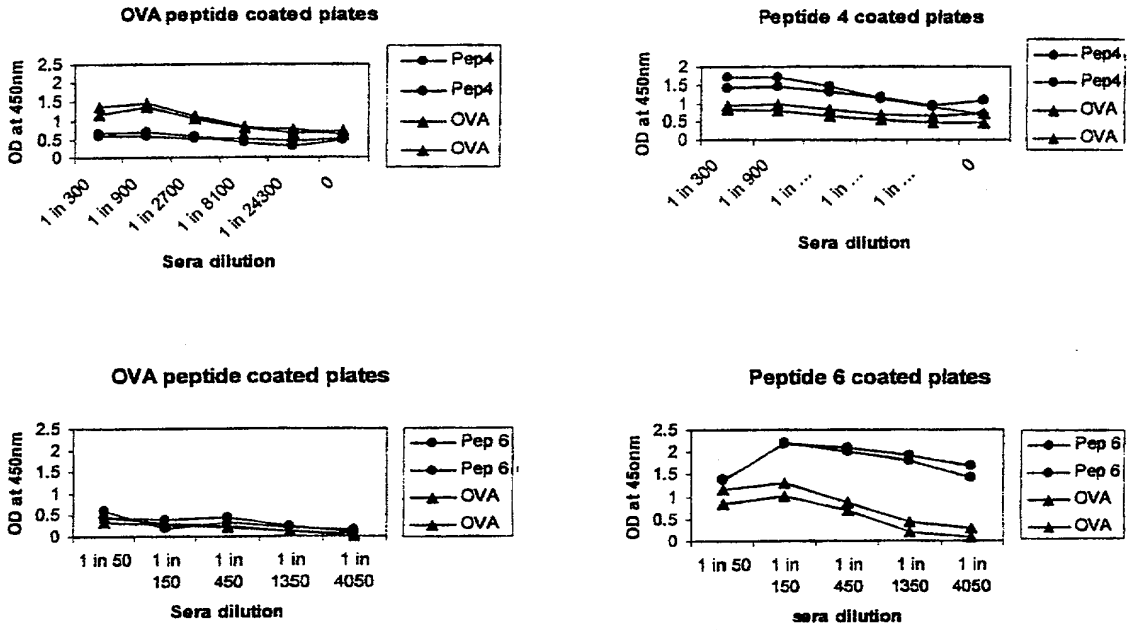
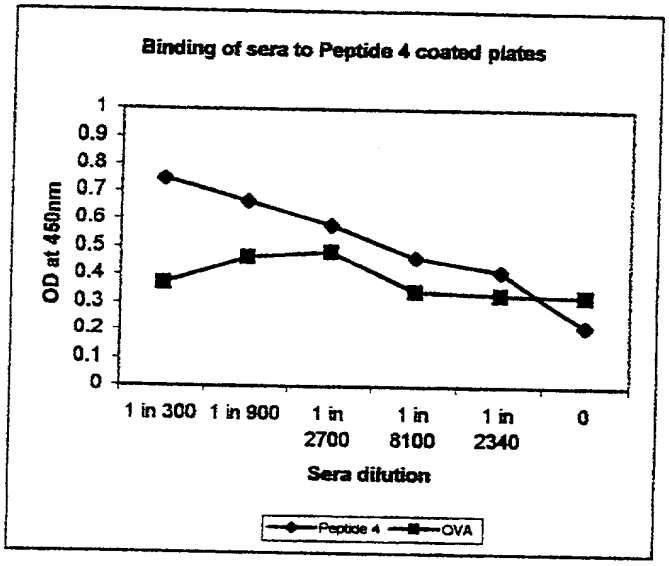

OVA peptide coated plates
OD at 450nm
2.5
2
1.5
1
0.5
0
1 in 300
1 in 900
1 in 2700
1 in 8100
1 in 24300
0
Sera dilution
Pep4
Pep4
OVA
OVA
Peptide 4 coated plates
OD at 450nm
2
1.5
1
0.5
0
1 in 300
1 in 900
1 in ...
1 in ...
1 in ...
0
Sera dilution
Pep4
Pep4
OVA
OVA
OVA peptide coated plates
OD at 450nm
2.5
2
1.5
1
0.5
0
1 in 50
1 in 150
1 in 450
1 in 1350
1 in 4050
Sera dilution
Pep 6
Pep 6
OVA
OVA
Peptide 6 coated plates
OD at 450nm
2.5
2
1.5
1
0.5
0
1 in 50
1 in 150
1 in 450
1 in 1350
1 in 4050
sera dilution
Pep 6
Pep 6
OVA
OVA

FIGURE 15a


Binding of sera to Peptide 4 coated plates
OD at 450nm
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 in 300
1 in 900
1 in 2700
1 in 8100
1 in 2340
0
Sera dilution
Peptide 4
OVA

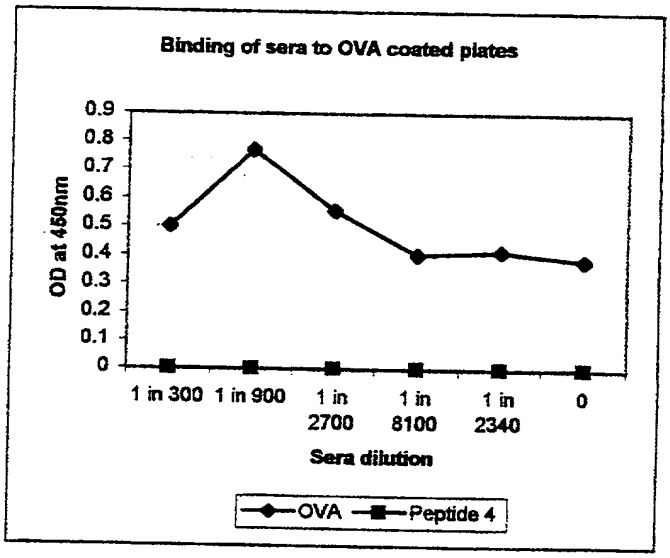
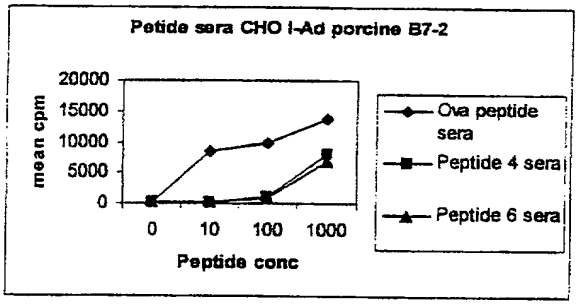
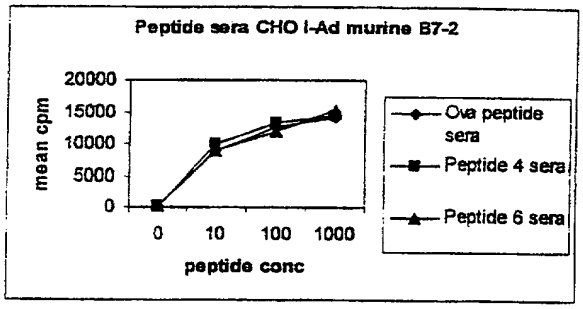

Binding of sera to OVA coated plates
OD at 450nm
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 in 300
1 in 900
1 in 2700
1 in 8100
1 in 2340
0
Sera dilution
OVA
Peptide 4

Figure 15b

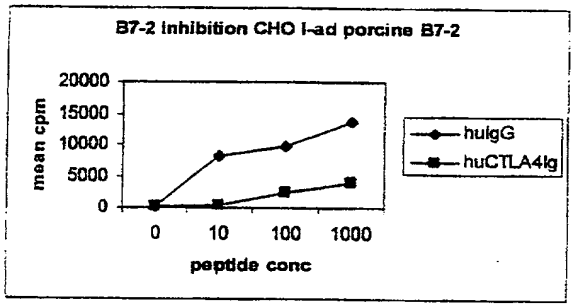
A



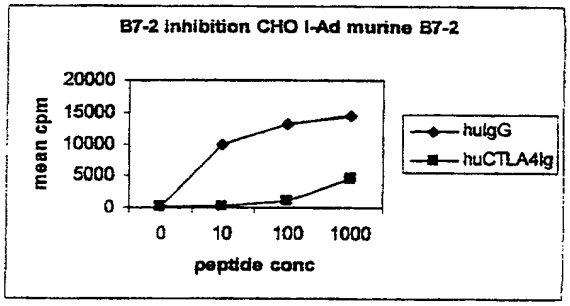
C



B



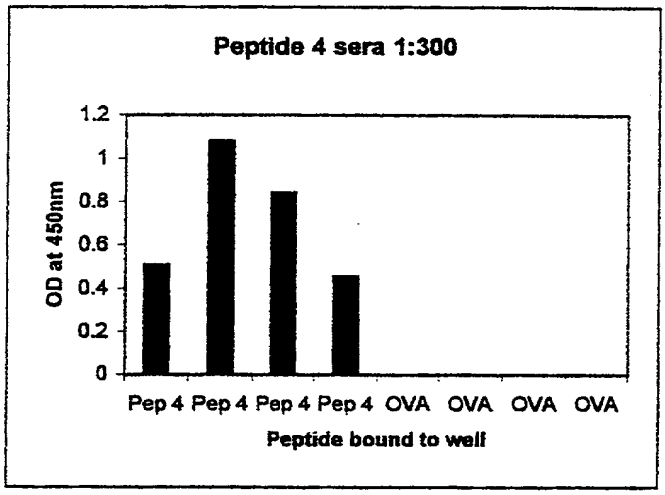
D



Porcine B7-2

Murine B7-2

Figure 1b


Peptide 4 sera 1:300
OD at 450nm
1.2
1
0.8
0.6
0.4
0.2
0
Pep 4 Pep 4 Pep 4 Pep 4 OVA OVA OVA OVA
Peptide bound to well

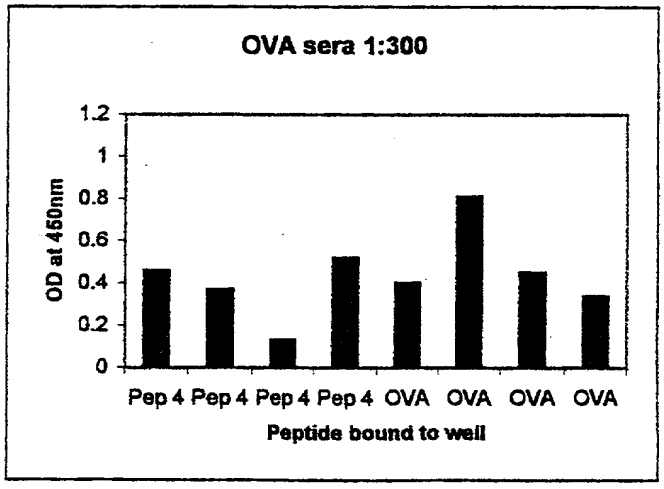

OVA sera 1:300
OD at 450nm
1.2
1
0.8
0.6
0.4
0.2
0
Pep 4 Pep 4 Pep 4 Pep 4 OVA OVA OVA OVA
Peptide bound to well

FIGURE 17a

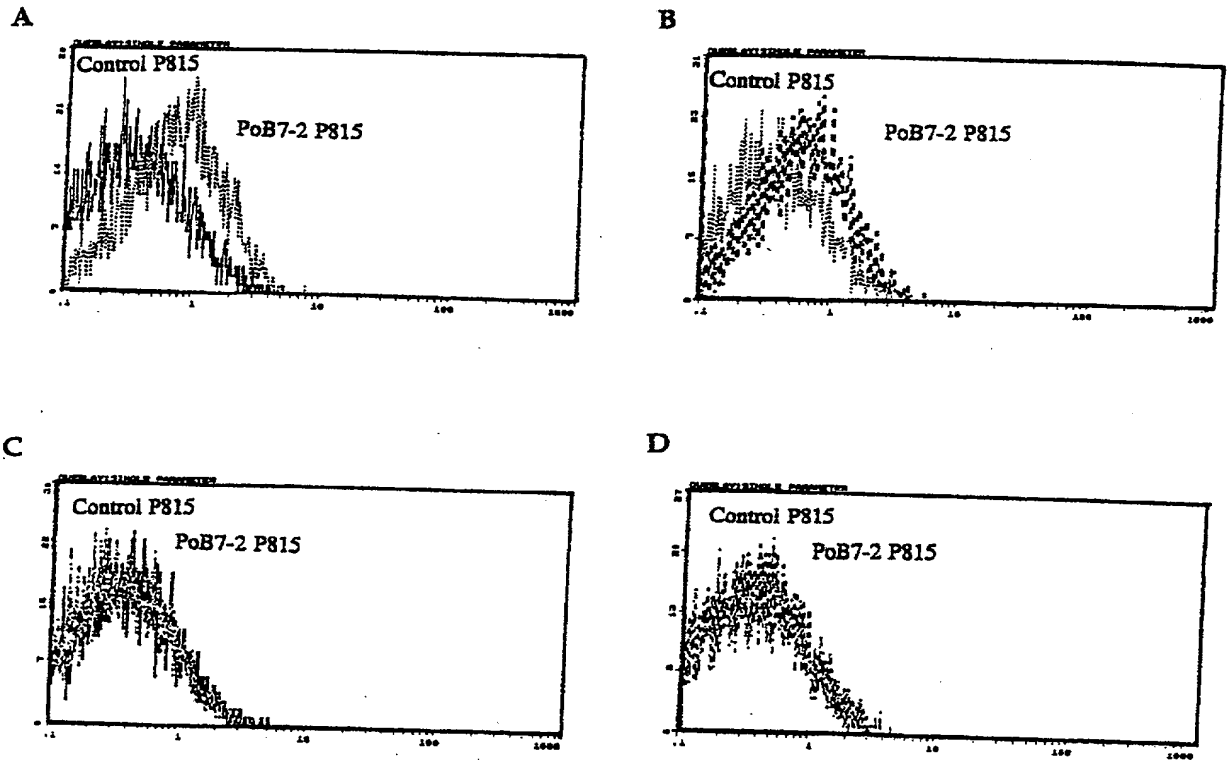
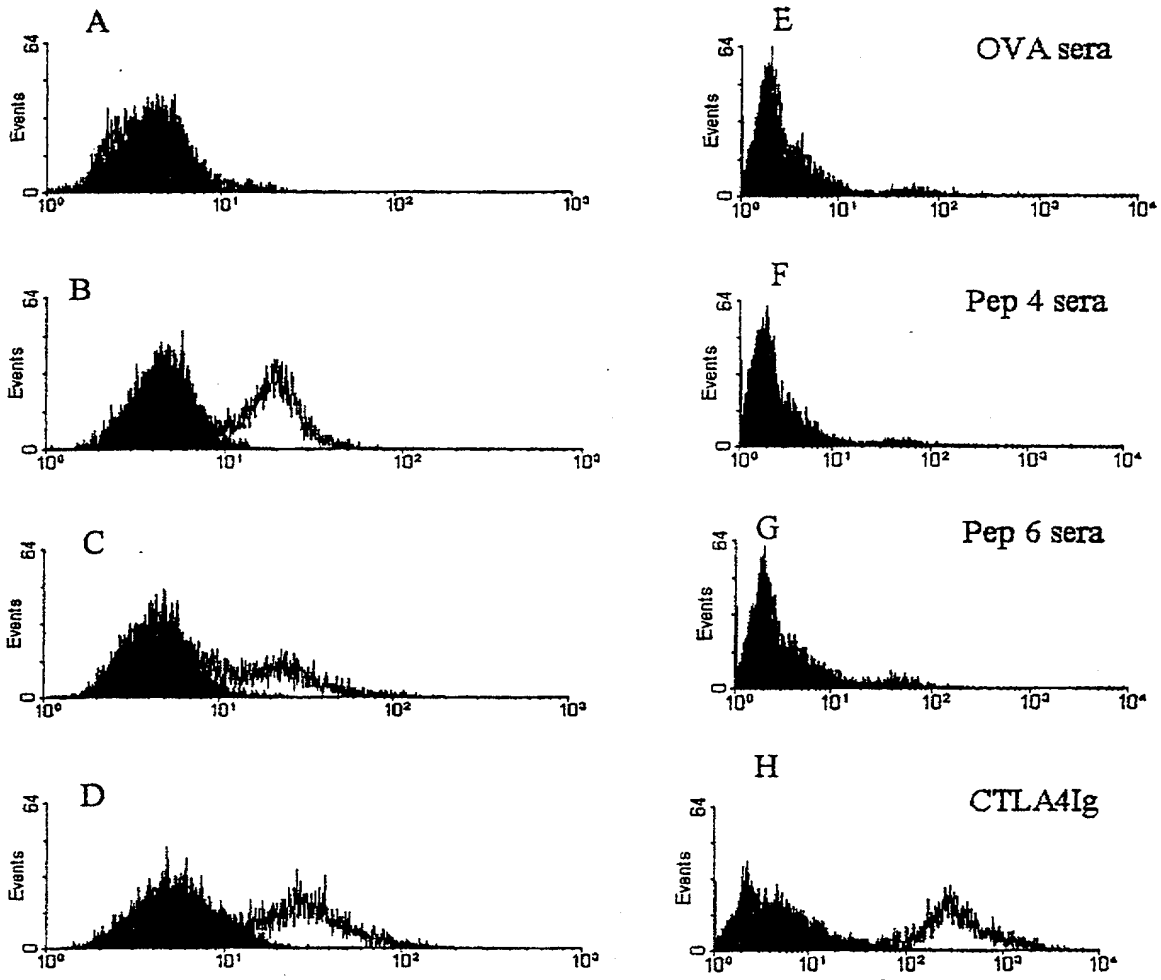

A
Control P815
PoB7-2 P815
B
Control P815
PoB7-2 P815
C
Control P815
PoB7-2 P815
D
Control P815
PoB7-2 P815

FIGURE 17b



Porcine CD86 Murine CD86

FIGURE 18

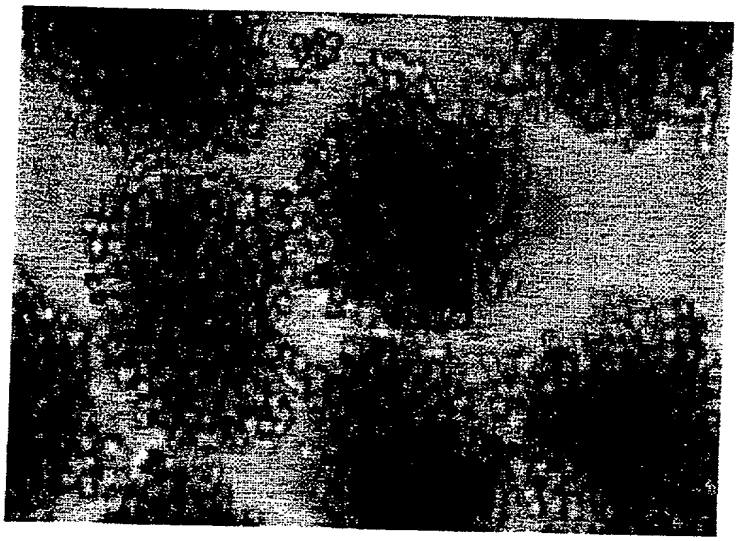


FIGURE 19

Day 1: Immunisation of C57BL-6 mice with whole ovalbumin (50 micrograms) in Complete freunds adjuvant (CFA)

↓

Day 14: First immunisation with chimeric peptide (100 micrograms) i.v.

Day 21: Second immunisation with chimeric peptide (100 micrograms) i.v.

Day 28: Third immunisation with chimeric peptide (100 micrograms) i.v.

↓

Day 32: Mice rendered diabetic by injection of streptozotocin i.p.

↓

Day 36 : Transplantation of 1000 porcine pancreatic islets under the kidney capsule of diabetic mice

↓

Day 37 onwards : Survival of islets assessed by measuring blood glucose levels

Figure 2o

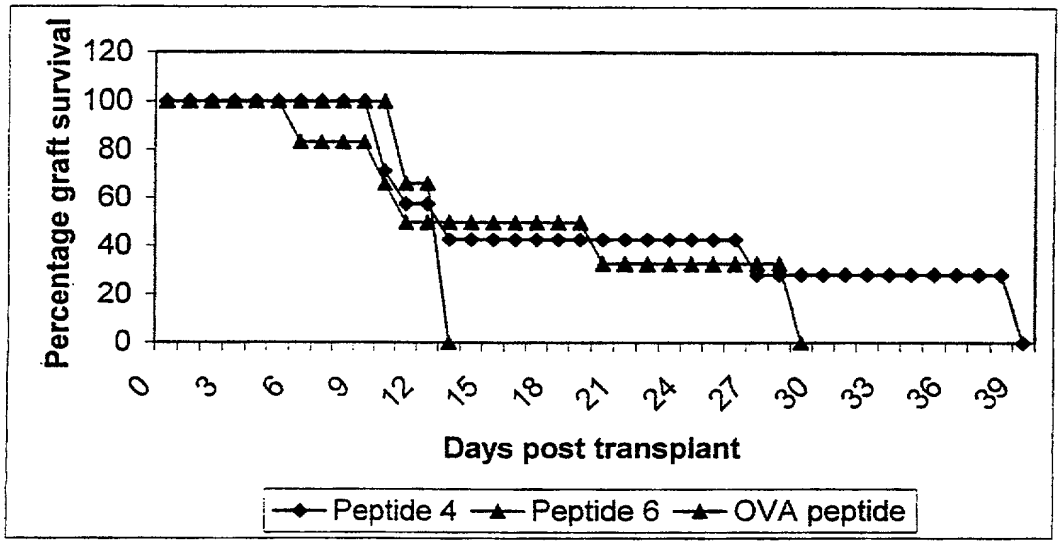

Percentage graft survival
120
100
80
60
40
20
0
0 3 6 9 12 15 18 21 24 27 30 33 36 39
Days post transplant
Peptide 4
Peptide 6
OVA peptide

FIGURE 21

poCD40protein(top), human CD40 protein(bottom)

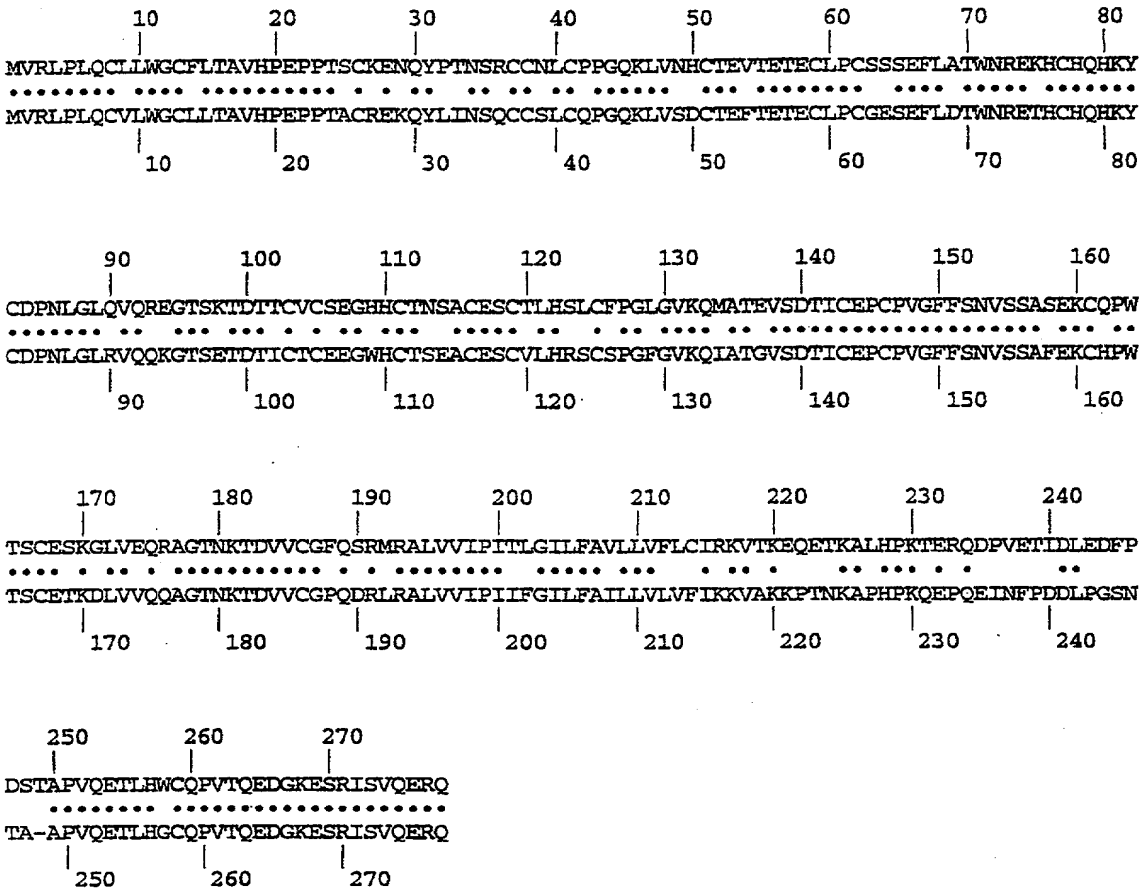


FIGURE 22

```
1    MVRLPLQCLL WGCFLTAVHP EPPTSCKENQ YPTNSRCCNL
41   CPPGQKLVNH CTEVTETECL PCSSSEFLAT WNREKHCHQH
81   KYCDPNLGLQ VQREGTSKTD TTCVCSEGHH CTNSACESCT
121  LHSLCFPGLG VKQMATEVSD TICEPCPVGF FSNVSSASEK
161  CQPWTSCESK GLVEQRAGTN KTDVVCGFQS RMRALVVIPI
201  TLGILFAVLL VFLCIRKVTK EQETKALHPK TERQDPVETI
241  DLEDFPDSTA PVQETLHWCQ PVTQEDGKES RISVQERQ
```

FIGURE 23

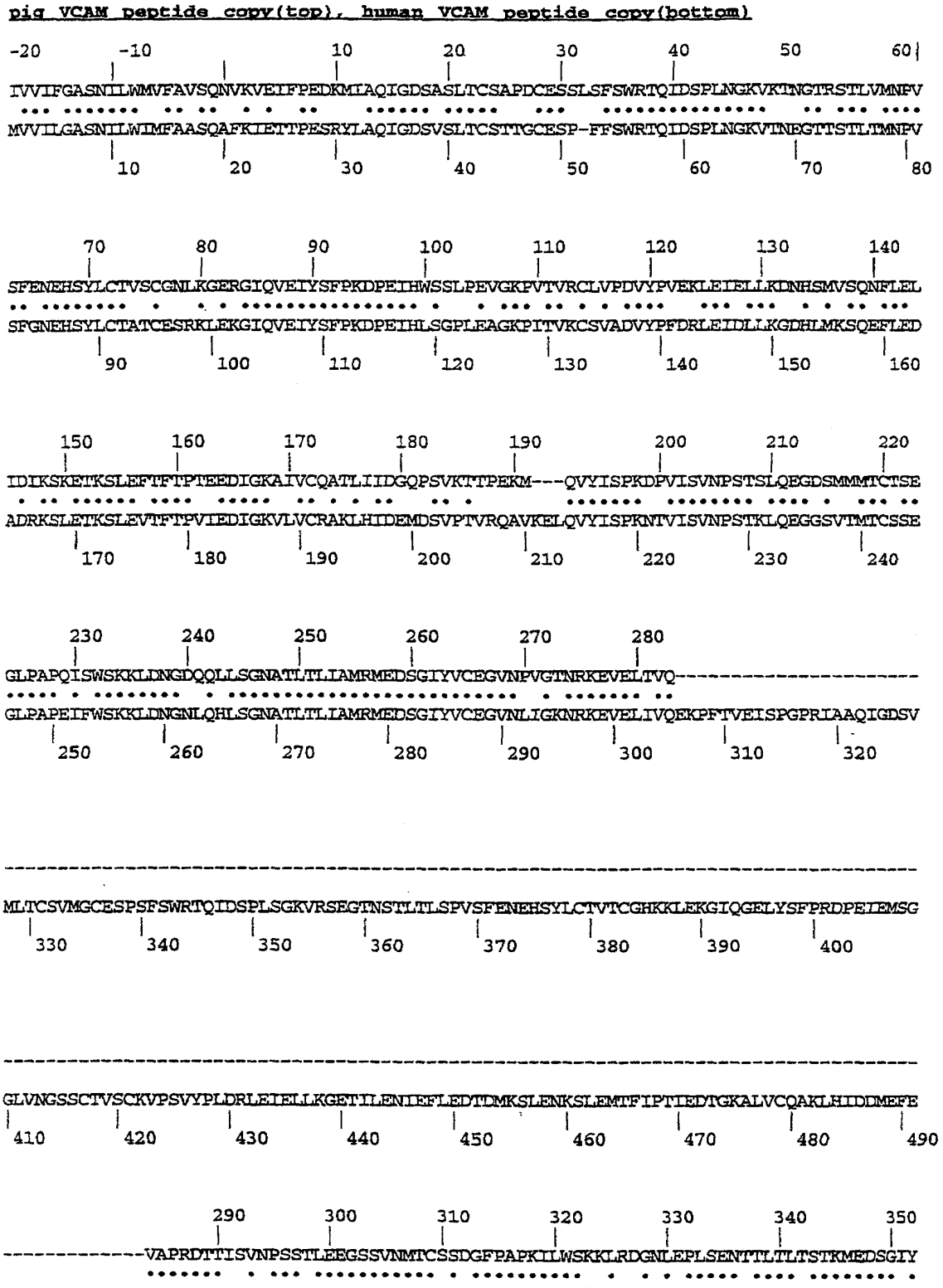


FIGURE 23-1

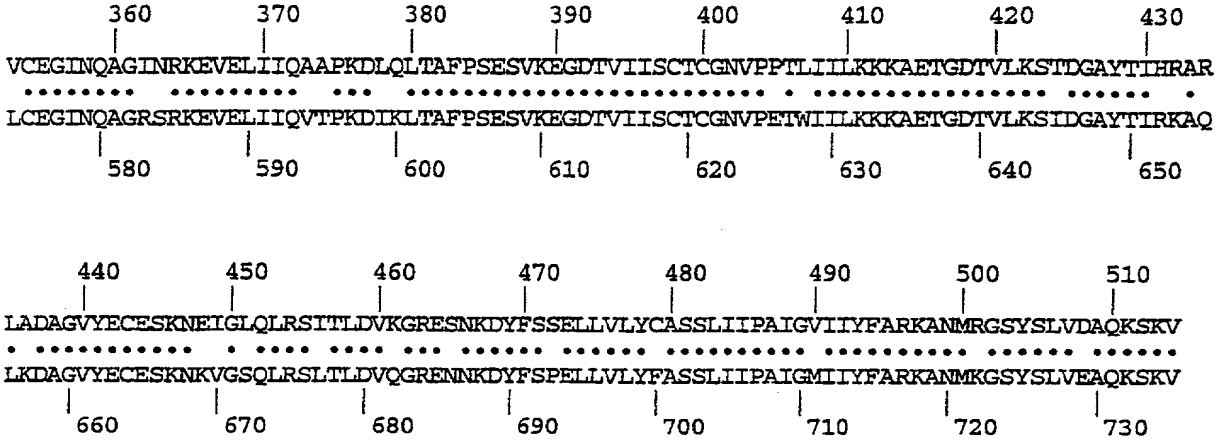

360 370 380 390 400 410 420 430
VCEGINQAGINRKEVELIIQAAPKDLQLTAFPSESVKEGDTVIISCTCGNVPPTLIILKKKAETGDTVLKSTDGAYTIHRAR
LCEGINQAGRSRKEVELIIQVTPKDIKLTAFPSESVKEGDTVIISCTCGNVPETWIILKKKAETGDTVLKSIDGAYTIRKAQ
580 590 600 610 620 630 640 650
440 450 460 470 480 490 500 510
LADAGVYECESKNEIGLQLRSITLDVKGRESNKDYFSSELLVLYCASSLIIPAIGVIIYFARKANMRGSYSLVDAQKSKV
LKDAGVYECESKNKVGSQLRSLTLDVQGRENNKDYFSPELLVLYFASSLIIPAIGMIIYFARKANMKGSYSLVEAQKSKV
660 670 680 690 700 710 720 730

FIGURE 24

↓ (signal sequence)

IVVIFGASNI LWMVFAVSQN VKVEIFPEDK MIAQIGDSAS

LTCSAPDCES SLSFSWRTQI DSPLNGKVKT NGTRSTLVMN

PVSFENEHSY LCTVSCGNLK GERGIQVEIY SFPKDPEIHW

SSLPEVGKPV TVRCLVPDVY PVEKLEIELL KDNHSMVSQN

FLELIDIKSK ETKSLEFTFT PTEEDIGKAI VCQATLIIDG

QPSVKTTPEK MQVYISPKDP VISVNPSTSL QEGDSMMMTC

TSEGLPAPQI SWSKKLDNGD QQLLSGNATL TLIAMRMEDS

GIYVCEGVNP VGTNRKEVEL TVQVAPRDTT ISVNPSSTLE

EGSSVNMTCS SDGFPAPKIL WSKKLRDGNL EPLSENTTLT

LTSTKMEDSG IYVCEGINQA GINRKEVELI IQAAPKDLQL

TAFPSESVKE GDTVIISCTC GNVPPTLIIL KKKAETGDTV

LKSTDGAYTI HRARLADAGV YECESKNEIG LQLRSITLDV

KGRESNKDYF SSELLVLYCA SSLIIPAIGV IIYFARKANM

RGSYSLVDAQ KSKV•

FIGURE 25

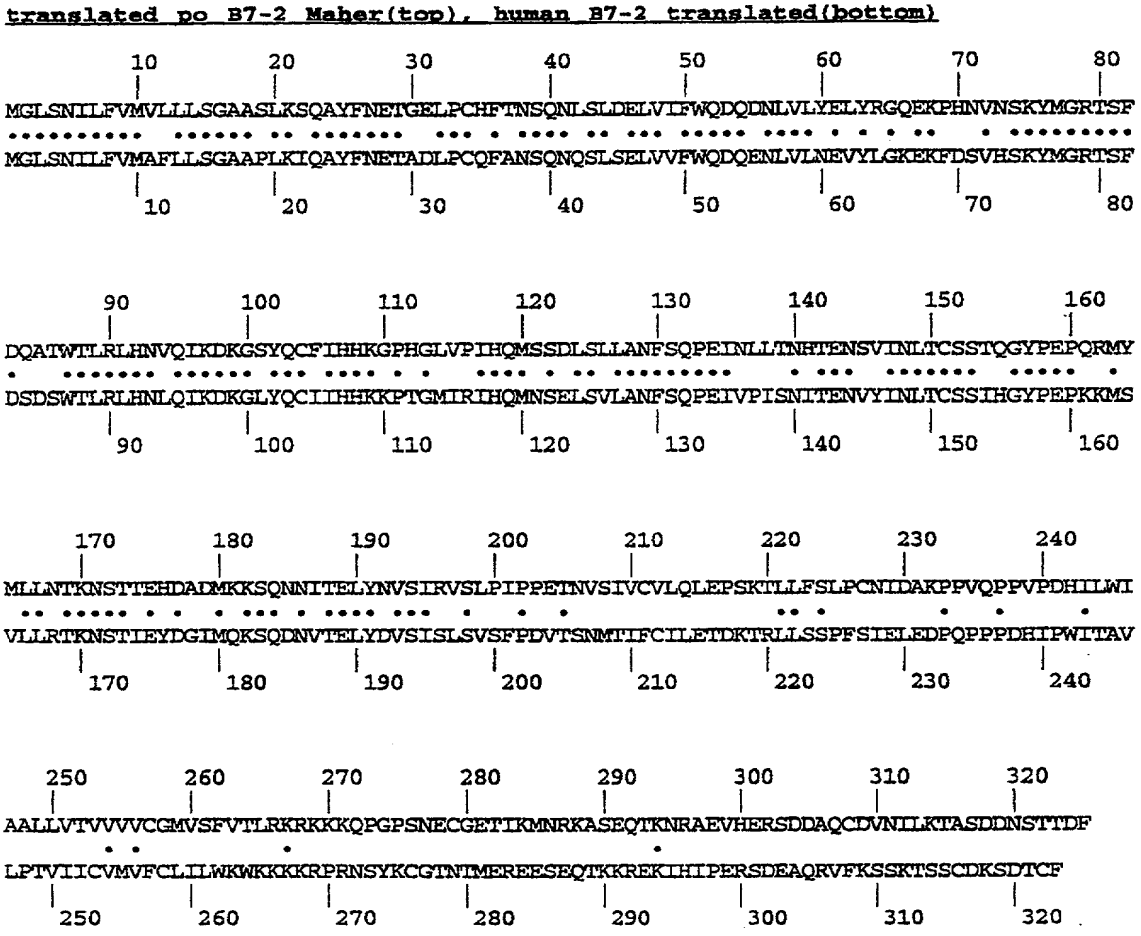

translated po B7-2 Maher(top), human B7-2 translated(bottom)
MGLSNILFVMVLLLSGAASLKSQAYFNETGELPCHFTNSQNLSLDELVLFWQDQDNLVLYELYRGQEKPHNVNSKYMGRTSF
MGLSNILFVMAFLLSGAAPLKIQAYFNETADLPCQFANSQNQSLSELVVFWQDQENLVLNEVYLGKEKFDSVHSKYMGRTSF
DQATWTLRLHNVQIKDKGSYQCFIHHKGPHGLVPIHQMSSDLSLLANFSQPEINLLTNHTENSVINLTCSSTQGYPEPQRMY
DSDSWTLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVLANFSQPEIVPISNITENVYINLTCSSIHGYPEPKKMS
MLLNTKNSTTEHDADMKKSQNNITELYNVSIRVSLPIPPETNVSIVCVLQLEPSKTLLFSLPCNIDAKPPVQPPVPDHILWI
VLLRTKNSTIEYDGIMQKSQDNVTELYDVSISLSVSFPDVTSNMTIFCILETDKTRLLSSPFSIELEDPQPPPDHIPWITAV
AALLVTVVVVCGMVSFVTLRKRKKKQPGPSNECGETIKMNRKASEQTKNRAEVHERSDDAQCDVNILKTASDDNSTTDF
LPTVIICVMVFCLILWKWKKKKRPRNSYKCGTNTMEREESEQTKKREKIHIPERSDEAQRVFKSSKTSSCDKSDTCF

FIGURE 26

```
1    MGLSNILFVM VLLLSGAASL KSQAYFNETG ELPCHFTNSQ

41   NLSLDELVIF WQDQDNLVLY ELYRGQEKPH NVNSKYMGRT

81   SFDQATWTLR LHNVQIKDKG SYQCFIHHKG PHGLVPIHQM

121  SSDLSLLANF SQPEINLLTN HTENSVINLT CSSTQGYPEP

161  QRMYMLLNTK NSTTEHDADM KKSQNNITEL YNVSIRVSLP

201  IPPETNVSIV CVLQLEPSKT LLFSLPCNID AKPPVQPPVP

241  DHILWIAALL VTVVVVCGMV SFVTLRKRKK KQPGPSNECG

281  ETIKMNRKAS EQTKNRAEVH ERSDDAQCDV NILKTASDDN

321  STTDF
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SEQUENCE LISTING

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<120> Immunosuppression

<130> P15700WO

<140> PCT/GB99/04200
<141> 1999-12-17

<150> 9827921.9
<151> 1998-12-19

<150> 9925015.1
<151> 1999-10-23

<160> 39

<170> PatentIn Ver. 2.1

<210> 1
<211> 288
<212> PRT
<213> Homo sapiens

<400> 1

```
Met Gly His Thr Arg Arg Gln Gly Thr Ser Pro Ser Lys Cys Pro Tyr
  1               5                  10                  15

Leu Asn Phe Phe Gln Leu Leu Val Leu Ala Gly Leu Ser His Phe Cys
             20                  25                  30

Ser Gly Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu
         35                  40                  45

Ser Cys Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile
     50                  55                  60

Tyr Trp Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp
 65                  70                  75                  80

Met Asn Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr
                 85                  90                  95

Asn Asn Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly
            100                 105                 110

Thr Tyr Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg
        115                 120                 125

Glu His Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr
    130                 135                 140

Pro Ser Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile
145                 150                 155                 160

Ile Cys Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu
                165                 170                 175

Glu Asn Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp
```

TMH:jlb 06/01/01 5585-59112 54103

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled IMPROVEMENT OF TOLERANCE TO A XENOGRAFT, the specification of which

☒ is attached hereto.

☐ was filed on _____ as United States Application No. _____.

☒ was filed on 17 December 1999 as International Application No. PCT/GB99/04200.

☐ and was amended on _____ (if applicable).

☐ with amendments through _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56. If this is a continuation-in-part application filed under the conditions specified in 35 U.S.C. § 120 which discloses and claims subject matter in addition to that disclosed in the prior copending application, I further acknowledge the duty to disclose material information as defined in 37 C.F.R. § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
9827921.9	United Kingdom	19 December 1998	☒	☐
9925015.1	United Kingdom	23 October 1999	☒	☐
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

I hereby claim the benefit under Title 35, United States Code,§ 119(e) of any United States provisional application(s) listed below:

Application Number	Filing Date

TMH:jlb 06/01/01 5585-59112 54103

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or § 365(c) of any PCT International application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT International filing date of this application:

PCT/GB99/04200	17 December 1999	Pending
(Application No.)	(Filing Date)	(Status: patented, Pending, abandoned)

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from _____ as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

I hereby appoint the practitioners associated with the customer number provided below to prosecute this application, to file a corresponding international application, and to transact all business in the Patent and Trademark Office connected therewith:

Customer Number 24197 KSCLW

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HAENDLER, Jeffrey B.	43,652	RUPERT, Wayne W.	34,420
HARDING, Tanya M.	42,630	RYBAK, Sheree L.	47,913
JAKUBEK, Joseph T.	34,190	SCOTTI, Robert F.	39,830
JONCUS, Stephen J.	44,809	SIEGEL, Susan Alpert	43,121
JONES, Michael D.	41,879	SLATER, Stacey C.	36,011
KLARQUIST, Kenneth S.	16,445	STEPHENS Jr., Donald L.	34,022
KLITZKE II, Ramon A.	30,188	STUART, John W.	24,540
LEIGH, James S.	20,434	VANDENBERG, John D.	31,312
MAURER, Gregory L.	43,781	WHINSTON, Arthur L.	19,155
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

TMH:jlb 06/01/01 5585-59112 54103

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Inventor's Signature [signature] 26.7.01
Date

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180 185 190

Pro Glu Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met
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Thr Thr Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg
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Val Asn Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro
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Ser Glu Ala Cys Glu Ser Cys Val Leu His Arg Ser Cys Ser Pro Gly
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145 150 155 160

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Ala Gly Thr Asn Lys Thr Asp Val Val Cys Gly Pro Gln Asp Arg Leu
180 185 190

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Leu Leu Val Leu Val Phe Ile Lys Lys Val Ala Lys Lys Pro Thr Asn
210 215 220

Lys Ala Pro His Pro Lys Gln Glu Pro Gln Glu Ile Asn Phe Pro Asp
225 230 235 240

Asp Leu Pro Gly Ser Asn Thr Ala Ala Pro Val Gln Glu Thr Leu His
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Gly Lys Val Thr Asn Glu Gly Thr Thr Ser Thr Leu Thr Met Asn Pro
65 70 75 80

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85 90 95

Ser Arg Lys Leu Glu Lys Gly Ile Gln Val Glu Ile Tyr Ser Phe Pro
100 105 110

Lys Asp Pro Glu Ile His Leu Ser Gly Pro Leu Glu Ala Gly Lys Pro
115 120 125

Ile Thr Val Lys Cys Ser Val Ala Asp Val Tyr Pro Phe Asp Arg Leu
130 135 140

Glu Ile Asp Leu Leu Lys Gly Asp His Leu Met Lys Ser Gln Glu Phe
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180 185 190

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195 200 205

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Gly Ile Asn Gln Ala Gly Arg Ser Arg Lys Glu Val Glu Leu Ile Ile
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Val Lys Glu Gly Asp Thr Val Ile Ile Ser Cys Thr Cys Gly Asn Val
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Val Leu Lys Ser Ile Asp Gly Ala Tyr Thr Ile Arg Lys Ala Gln Leu
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Lys Asp Ala Gly Val Tyr Glu Cys Glu Ser Lys Asn Lys Val Gly Ser
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Asp Tyr Phe Ser Pro Glu Leu Leu Val Leu Tyr Phe Ala Ser Ser Leu
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Ile Ile Pro Ala Ile Gly Met Ile Ile Tyr Phe Ala Arg Lys Ala Asn
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Gln Leu Ser Lys Ser Val Lys Asp Lys Val Leu Leu Pro Cys Arg Tyr
     50                  55                  60

Asn Ser Pro His Glu Asp Glu Ser Glu Asp Arg Ile Tyr Trp Gln Lys
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His Asp Lys Val Val Leu Ser Val Ile Ala Gly Lys Leu Lys Val Trp
                 85                  90                  95

Pro Glu Tyr Lys Asn Arg Thr Leu Tyr Asp Asn Thr Thr Tyr Ser Leu
            100                 105                 110

Ile Ile Leu Gly Leu Val Leu Ser Asp Arg Gly Thr Tyr Ser Cys Val
        115                 120                 125

Val Gln Lys Lys Glu Arg Gly Thr Tyr Glu Val Lys His Leu Ala Leu
    130                 135                 140

Val Lys Leu Ser Ile Lys Ala Asp Phe Ser Thr Pro Asn Ile Thr Glu
145                 150                 155                 160

Ser Gly Asn Pro Ser Ala Asp Thr Lys Arg Ile Thr Cys Phe Ala Ser
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Gly Gly Phe Pro Lys Pro Arg Phe Ser Trp Leu Glu Asn Gly Arg Glu
            180                 185                 190

Leu Pro Gly Ile Asn Thr Thr Ile Ser Gln Asp Pro Glu Ser Glu Leu
        195                 200                 205

Tyr Thr Ile Ser Ser Gln Leu Asp Phe Asn Thr Thr Arg Asn His Thr
    210                 215                 220

Ile Lys Cys Leu Ile Lys Tyr Gly Asp Ala His Val Ser Glu Asp Phe
225                 230                 235                 240

Thr Trp Glu Lys Pro Pro Glu Asp Pro Pro Asp Ser Lys Asn Thr Leu
                245                 250                 255

Val Leu Phe Gly Ala Gly Phe Gly Ala Val Ile Thr Val Val Val Ile
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Val Val Ile Ile Lys Cys Phe Cys Lys His Arg Ser Cys Phe Arg Arg
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        35   40   45

Ser Leu Ser Glu Leu Val Val Phe Trp Gln Asp Gln Gln Lys Leu Val
    50   55   60

Leu Tyr Glu His Tyr Leu Gly Thr Glu Lys Leu Asp Ser Val Asn Ala
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Lys Tyr Leu Gly Arg Thr Ser Phe Asp Arg Asn Asn Trp Thr Leu Arg
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Leu His Asn Val Gln Ile Lys Asp Met Gly Ser Tyr Asp Cys Phe Ile
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Gln Lys Lys Pro Pro Thr Gly Ser Ile Ile Leu Gln Gln Thr Leu Thr
        115   120   125

Glu Leu Ser Val Ile Ala Asn Phe Ser Glu Pro Glu Ile Lys Leu Ala
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Gln Asn Val Thr Gly Asn Ser Gly Ile Asn Leu Thr Cys Thr Ser Lys
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Gln Gly His Pro Lys Pro Lys Lys Met Tyr Phe Leu Ile Thr Asn Ser
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Thr Asn Glu Tyr Gly Asp Asn Met Gln Ile Ser Gln Asp Asn Val Thr
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Glu Leu Phe Ser Ile Ser Asn Ser Leu Ser Leu Ser Phe Pro Asp Gly
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Thr Tyr Trp Lys Glu Ile Thr Ala Ser Val Thr Val Ala Leu Leu Leu
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Val Met Leu Leu Ile Ile Val Cys His Lys Lys Pro Asn Gln Pro Ser
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         35                  40                  45

Ser His Cys Thr Ala Leu Glu Lys Thr Gln Cys His Pro Cys Asp Ser
     50                  55                  60

Gly Glu Phe Ser Ala Gln Trp Asn Arg Glu Ile Arg Cys His Gln His
 65                  70                  75                  80

Arg His Cys Glu Pro Asn Gln Gly Leu Arg Val Lys Lys Glu Gly Thr
                 85                  90                  95

Ala Glu Ser Asp Thr Val Cys Thr Cys Lys Glu Gly Gln His Cys Thr
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Ser Lys Asp Cys Glu Ala Cys Ala Gln His Thr Pro Cys Ile Pro Gly
        115                 120                 125

Phe Gly Val Met Glu Met Ala Thr Glu Thr Thr Asp Thr Val Cys His
    130                 135                 140

Pro Cys Pro Val Gly Phe Phe Ser Asn Gln Ser Ser Leu Phe Glu Lys
145                 150                 155                 160

Cys Tyr Pro Trp Thr Ser Cys Glu Asp Lys Asn Leu Glu Val Leu Gln
                165                 170                 175

Lys Gly Thr Ser Gln Thr Asn Val Ile Cys Gly Leu Lys Ser Arg Met
            180                 185                 190

Arg Ala Leu Leu Val Ile Pro Val Val Met Gly Ile Leu Ile Thr Ile
        195                 200                 205

Phe Gly Val Phe Leu Tyr Ile Lys Lys Val Val Lys Lys Pro Lys Asp
    210                 215                 220

Asn Glu Met Leu Pro Pro Ala Ala Arg Arg Gln Asp Pro Gln Glu Met
225                 230                 235                 240

Glu Asp Tyr Pro Gly His Asn Thr Ala Ala Pro Val Gln Glu Thr Leu
                245                 250                 255

His Gly Cys Gln Pro Val Thr Gln Glu Asp Gly Lys Glu Ser Arg Ile
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Ser Val Gln Glu Arg Gln Val Thr Asp Ser Ile Ala Leu Arg Pro Leu
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<212> DNA
<213> Porcus spp

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ctgcttttct ccctaccttg taatatagat gcaaagccac ctgtgcaacc ccctgtccca 720
gaccacatcc tctggattgc agctctactt gtaacagtgg tcgttgtgtg tgggatggtg 780
tcctttgtaa cactaaggaa aaggaagaag aagcagcctg gcccctctaa tgaatgtggt 840
gaaaccatca aaatgaacag gaaggcgagt gaacaaacta agaacagagc agaagtccat 900
gaacgatctg atgatgccca gtgtgatgtt aatattttaa agacagcctc agatgacaac 960
agtactacag atttttaatt aaagagtaaa ctcc 994

<210> 14
<211> 330
<212> PRT
<213> Porcus spp

<400> 14
Met Gly Leu Ser Asn Ile Leu Phe Val Met Val Leu Leu Leu Ser Gly
1 5 10 15

Ala Ala Ser Leu Lys Ser Gln Ala Tyr Phe Asn Glu Thr Gly Glu Leu
20 25 30

Pro Cys His Phe Thr Asn Ser Gln Asn Leu Ser Leu Asp Glu Leu Val
35 40 45

Ile Phe Trp Gln Asp Gln Asp Asn Leu Val Leu Tyr Glu Leu Tyr Arg
50 55 60

Gly Gln Glu Lys Pro His Asn Val Asn Ser Lys Tyr Met Gly Arg Thr
65 70 75 80

Ser Phe Asp Gln Ala Thr Trp Thr Leu Arg Leu His Asn Val Gln Ile
85 90 95

Lys Asp Lys Gly Ser Tyr Gln Cys Phe Ile His His Lys Gly Pro His
100 105 110

Gly Leu Val Pro Ile His Gln Met Ser Ser Asp Leu Ser Leu Leu Ala
115 120 125

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Asn Phe Ser Gln Pro Glu Ile Asn Leu Leu Thr Asn His Thr Glu Asn
    130                 135                 140

Ser Val Ile Asn Leu Thr Cys Ser Ser Thr Gln Gly Tyr Pro Glu Pro
145                 150                 155                 160

Gln Arg Met Tyr Met Leu Leu Asn Thr Lys Asn Ser Thr Thr Glu His
                165                 170                 175

Asp Ala Asp Met Lys Lys Ser Gln Asn Asn Ile Thr Glu Leu Tyr Asn
            180                 185                 190

Val Ser Ile Arg Val Ser Leu Pro Ile Pro Pro Glu Thr Asn Val Ser
        195                 200                 205

Ile Val Cys Val Leu Gln Leu Glu Pro Ser Lys Thr Leu Leu Phe Ser
    210                 215                 220

Leu Pro Cys Asn Ile Asp Ala Lys Pro Pro Val Gln Pro Pro Val Pro
225                 230                 235                 240

Asp His Ile Leu Trp Ile Ala Ala Leu Leu Val Thr Val Val Val Val
                245                 250                 255

Cys Gly Met Val Ser Phe Val Thr Leu Arg Lys Arg Lys Lys Lys Gln
            260                 265                 270

Pro Gly Pro Ser Asn Glu Cys Gly Glu Thr Ile Lys Met Asn Arg Lys
        275                 280                 285

Ala Ser Glu Gln Thr Lys Asn Arg Ala Glu Val His Glu Arg Ser Asp
    290                 295                 300

Asp Ala Gln Cys Asp Val Asn Ile Leu Lys Thr Ala Ser Asp Asp Asn
305                 310                 315                 320

Ser Thr Thr Asp Phe Leu Lys Ser Lys Leu
                325                 330
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<210> 15
<211> 837
<212> DNA
<213> Porcus

<400> 15

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gaaccaccca cttcatgcaa agaaaaccaa tacccaacaa acagccggtg ctgtaatttg 120
tgcccgccag gacagaaact ggtgaaccac tgcacagagg tcactgaaac agaatgcctt 180
ccttgcagtt ccagcgaatt cctagccacc tggaatagag agaaacactg tcatcagcac 240
aaatactgcg accccaacct aggtctccag gtccagaggg agggcacctc gaaaacagac 300
accacttgtg tgtgcagtga aggccatcac tgtaccaaca gcgcctgtga aagttgcacc 360
ttgcacagct tgtgcttccc tggcctcggg gtcaagcaga tggcgacaga ggtttctgac 420
actatctgtg aaccctgccc agttggcttc ttctccaatg tatcatctgc ttcagaaaag 480
tgtcagcctt ggacaagctg cgagagcaaa ggcctggtgg aacaacgtgc ggggactaac 540
aagaccgatg ttgtctgtgg tttccagagt cggatgagag ccctggtggt tatccccatc 600
acgctgggga tcctgtttgc cgtcctgttg gtatttctct gtatcagaaa ggtgaccaag 660
gagcaggaga ctaaggccct gcaccctaag actgaaaggc aggatcccgt ggagacgatt 720
gatctggagg attttcccga ctccaccgct ccggtgcagg agaccttaca ttggtgccag 780
cccgtcaccc aggaggacgg caaagagagt cgcatctcag tgcaggagag acagtga    837
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<210> 16
<211> 278
<212> PRT
<213> Porcus

<400> 16
Met Val Arg Leu Pro Leu Gln Cys Leu Leu Trp Gly Cys Phe Leu Thr
1 5 10 15

Ala Val His Pro Glu Pro Pro Thr Ser Cys Lys Glu Asn Gln Tyr Pro
20 25 30

Thr Asn Ser Arg Cys Cys Asn Leu Cys Pro Pro Gly Gln Lys Leu Val
35 40 45

Asn His Cys Thr Glu Val Thr Glu Thr Glu Cys Leu Pro Cys Ser Ser
50 55 60

Ser Glu Phe Leu Ala Thr Trp Asn Arg Glu Lys His Cys His Gln His
65 70 75 80

Lys Tyr Cys Asp Pro Asn Leu Gly Leu Gln Val Gln Arg Glu Gly Thr
85 90 95

Ser Lys Thr Asp Thr Thr Cys Val Cys Ser Glu Gly His His Cys Thr
100 105 110

Asn Ser Ala Cys Glu Ser Cys Thr Leu His Ser Leu Cys Phe Pro Gly
115 120 125

Leu Gly Val Lys Gln Met Ala Thr Glu Val Ser Asp Thr Ile Cys Glu
130 135 140

Pro Cys Pro Val Gly Phe Phe Ser Asn Val Ser Ser Ala Ser Glu Lys
145 150 155 160

Cys Gln Pro Trp Thr Ser Cys Glu Ser Lys Gly Leu Val Glu Gln Arg
165 170 175

Ala Gly Thr Asn Lys Thr Asp Val Val Cys Gly Phe Gln Ser Arg Met
180 185 190

Arg Ala Leu Val Val Ile Pro Ile Thr Leu Gly Ile Leu Phe Ala Val
195 200 205

Leu Leu Val Phe Leu Cys Ile Arg Lys Val Thr Lys Glu Gln Glu Thr
210 215 220

Lys Ala Leu His Pro Lys Thr Glu Arg Gln Asp Pro Val Glu Thr Ile
225 230 235 240

Asp Leu Glu Asp Phe Pro Asp Ser Thr Ala Pro Val Gln Glu Thr Leu
245 250 255

His Trp Cys Gln Pro Val Thr Gln Glu Asp Gly Lys Glu Ser Arg Ile
260 265 270

Ser Val Gln Glu Arg Gln
275

<210> 17
<211> 534
<212> PRT
<213> Porcus

<400> 17
Ile Val Val Ile Phe Gly Ala Ser Asn Ile Leu Trp Met Val Phe Ala
1   5   10   15

Val Ser Gln Asn Val Lys Val Glu Ile Phe Pro Glu Asp Lys Met Ile
            20   25   30

Ala Gln Ile Gly Asp Ser Ala Ser Leu Thr Cys Ser Ala Pro Asp Cys
        35   40   45

Glu Ser Ser Leu Ser Phe Ser Trp Arg Thr Gln Ile Asp Ser Pro Leu
    50   55   60

Asn Gly Lys Val Lys Thr Asn Gly Thr Arg Ser Thr Leu Val Met Asn
65   70   75   80

Pro Val Ser Phe Glu Asn Glu His Ser Tyr Leu Cys Thr Val Ser Cys
                85   90   95

Gly Asn Leu Lys Gly Glu Arg Gly Ile Gln Val Glu Ile Tyr Ser Phe
            100   105   110

Pro Lys Asp Pro Glu Ile His Trp Ser Ser Leu Pro Glu Val Gly Lys
        115   120   125

Pro Val Thr Val Arg Cys Leu Val Pro Asp Val Tyr Pro Val Glu Lys
    130   135   140

Leu Glu Ile Glu Leu Leu Lys Asp Asn His Ser Met Val Ser Gln Asn
145   150   155   160

Phe Leu Glu Leu Ile Asp Ile Lys Ser Lys Glu Thr Lys Ser Leu Glu
                165   170   175

Phe Thr Phe Thr Pro Thr Glu Glu Asp Ile Gly Lys Ala Ile Val Cys
            180   185   190

Gln Ala Thr Leu Ile Ile Asp Gly Gln Pro Ser Val Lys Thr Thr Pro
        195   200   205

Glu Lys Met Gln Val Tyr Ile Ser Pro Lys Asp Pro Val Ile Ser Val
    210   215   220

Asn Pro Ser Thr Ser Leu Gln Glu Gly Asp Ser Met Met Met Thr Cys
225   230   235   240

Thr Ser Glu Gly Leu Pro Ala Pro Gln Ile Ser Trp Ser Lys Lys Leu
                245   250   255

Asp Asn Gly Asp Gln Gln Leu Leu Ser Gly Asn Ala Thr Leu Thr Leu
            260   265   270

Ile Ala Met Arg Met Glu Asp Ser Gly Ile Tyr Val Cys Glu Gly Val
        275   280   285

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Asn Pro Val Gly Thr Asn Arg Lys Glu Val Glu Leu Thr Val Gln Val
    290                 295                 300

Ala Pro Arg Asp Thr Thr Ile Ser Val Asn Pro Ser Ser Thr Leu Glu
305                 310                 315                 320

Glu Gly Ser Ser Val Asn Met Thr Cys Ser Ser Asp Gly Phe Pro Ala
                325                 330                 335

Pro Lys Ile Leu Trp Ser Lys Lys Leu Arg Asp Gly Asn Leu Glu Pro
            340                 345                 350

Leu Ser Glu Asn Thr Thr Leu Thr Leu Thr Ser Thr Lys Met Glu Asp
        355                 360                 365

Ser Gly Ile Tyr Val Cys Glu Gly Ile Asn Gln Ala Gly Ile Asn Arg
    370                 375                 380

Lys Glu Val Glu Leu Ile Ile Gln Ala Ala Pro Lys Asp Leu Gln Leu
385                 390                 395                 400

Thr Ala Phe Pro Ser Glu Ser Val Lys Glu Gly Asp Thr Val Ile Ile
                405                 410                 415

Ser Cys Thr Cys Gly Asn Val Pro Pro Thr Leu Ile Ile Leu Lys Lys
            420                 425                 430

Lys Ala Glu Thr Gly Asp Thr Val Leu Lys Ser Thr Asp Gly Ala Tyr
        435                 440                 445

Thr Ile His Arg Ala Arg Leu Ala Asp Ala Gly Val Tyr Glu Cys Glu
    450                 455                 460

Ser Lys Asn Glu Ile Gly Leu Gln Leu Arg Ser Ile Thr Leu Asp Val
465                 470                 475                 480

Lys Gly Arg Glu Ser Asn Lys Asp Tyr Phe Ser Ser Glu Leu Leu Val
                485                 490                 495

Leu Tyr Cys Ala Ser Ser Leu Ile Ile Pro Ala Ile Gly Val Ile Ile
            500                 505                 510

Tyr Phe Ala Arg Lys Ala Asn Met Arg Gly Ser Tyr Ser Leu Val Asp
        515                 520                 525

Ala Gln Lys Ser Lys Val
    530
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<210> 18
<211> 807
<212> DNA
<213> Vacca spp

<400> 18
atggttcgtt tgccactgca gtgtctcttc tggggcttct ttctgaccgc cgtccactca 60
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tgcccgccgg gacagaaact ggtgaacgac tgcacagagg tcagcaaaac agaatgccag 180
tcctgcggta aaggcgaatt cttgtccacc tggaacagag agaaatactg tcacgagcac 240
agatactgca accccaacct agggctccgg atccagagcg agggtacctt gaatacagac 300
accatttgtg tatgtgtcga aggccaacac tgtaccagtc acacctgcga aagttgcacg 360

ccccacagct tgtgtctccc tggcttcggg gtcaagcaga tcgctacagg gcttttggat 420
accgtctgtg aaccctgccc gctcggcttc ttctccaacg tgtcatctgc ttttgaaaag 480
tgtcaccgtt ggacaagctg cgagagaaaa ggcctggtgg aacaacacgt ggggacgaac 540
aagacagatg ttgtctgcgg tttccagagt cggatgagga ccctggtggt gatccccgtc 600
acgatgggag tcttgtttgc tgtcctgttg gtatctgcct gtatcaggaa cataaccaag 660
aagcggcagc taaggccctg caccctatgg ctgaaaggca ggatcccgtg gagacgattg 720
atccggagga ttttcccggc ccccacccgc ctctccggtg caagagacct tatgctggtg 780
tcagccggtc gcccaggagg acggcaa 807

<210> 19
<211> 269
<212> PRT
<213> Vacca spp

<400> 19
Met Val Arg Leu Pro Leu Gln Cys Leu Phe Trp Gly Phe Phe Leu Thr
1 5 10 15

Ala Val His Ser Glu Pro Ala Thr Ala Cys Gly Glu Lys Gln Tyr Pro
20 25 30

Val Asn Ser Leu Cys Cys Asp Leu Cys Pro Pro Gly Gln Lys Leu Val
35 40 45

Asn Asp Cys Thr Glu Val Ser Lys Thr Glu Cys Gln Ser Cys Gly Lys
50 55 60

Gly Glu Phe Leu Ser Thr Trp Asn Arg Glu Lys Tyr Cys His Glu His
65 70 75 80

Arg Tyr Cys Asn Pro Asn Leu Gly Leu Arg Ile Gln Ser Glu Gly Thr
85 90 95

Leu Asn Thr Asp Thr Ile Cys Val Cys Val Glu Gly Gln His Cys Thr
100 105 110

Ser His Thr Cys Glu Ser Cys Thr Pro His Ser Leu Cys Leu Pro Gly
115 120 125

Phe Gly Val Lys Gln Ile Ala Thr Gly Leu Leu Asp Thr Val Cys Glu
130 135 140

Pro Cys Pro Leu Gly Phe Phe Ser Asn Val Ser Ser Ala Phe Glu Lys
145 150 155 160

Cys His Arg Trp Thr Ser Cys Glu Arg Lys Gly Leu Val Glu Gln His
165 170 175

Val Gly Thr Asn Lys Thr Asp Val Val Cys Gly Phe Gln Ser Arg Met
180 185 190

Arg Thr Leu Val Val Ile Pro Val Thr Met Gly Val Leu Phe Ala Val
195 200 205

Leu Leu Val Ser Ala Cys Ile Arg Asn Ile Thr Lys Lys Arg Gln Leu
210 215 220

Arg Pro Cys Thr Leu Trp Leu Lys Gly Arg Ile Pro Trp Arg Arg Leu
225 230 235 240

Ile Arg Arg Ile Phe Pro Ala Pro Thr Arg Leu Ser Gly Ala Arg Asp
245 250 255

Leu Met Leu Val Ser Ala Gly Arg Pro Gly Gly Arg Gln
260 265

<210> 20
<211> 867
<212> DNA
<213> Vacca spp

<400> 20
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gaagtgaaag aagtggcaac gctgtcctgt ggtcacaatg tttctgttga agagctggca 180
caaactcgca tctactggca aaaggagaag aaaatggtgc tgactatgat gtctggggac 240
atgaatatat ggcccgagta caagaaccgg accatctttg atatcactaa taacctctcc 300
attgtgatcc tggctctgcg cccatctgac gagggcacat acgagtgtgt tgttctgaag 360
tatgaaaaag acgctttcaa gcgggaacac ctggctgaag tgacgttatc agtcaaagct 420
gacttcccta cacctagtat atctgacttt gaaattccaa cttctaatat tagaaggata 480
atttgctcaa cctctggagg ttttccagag cctcacctct cctggttgga aaatggagaa 540
gaattaaatg ccatcaacac aacagtttcc caagatcctg aaactgagct ctatgctgtt 600
agcagcaaac tggatttcaa tatgacaacc aaccacagct tcatgtgtct catcaagtat 660
ggacatttaa gagtgaatca gaccttcaac tggaatacaa ccaagcaaga gcattttcct 720
gataacctgc tcccatcctg ggccattacc ttaatctcag taaatggaat ttttgtgata 780
tgctgcctga cctactgctt tgccccaaga tgcagagaga gaaggaggaa tgagagattg 840
agaagggaaa gtgtacgccc tgtataa 867

<210> 21
<211> 35
<212> DNA
<213> Porcus spp

<400> 21
gcatggatcc atgggactga gtaacattct ctttg 35

<210> 22
<211> 34
<212> DNA
<213> Porcus

<400> 22
gcatgtcgac ttaaaaatct gtagtactgt tgtc 34

<210> 23
<211> 17
<212> DNA
<213> Porcus

<400> 23
agaccgtctt cctttag 17

<210> 24
<211> 21
<212> DNA
<213> Porcus

<400> 24
ttggatcctc catgttatcc c 21

<210> 25
<211> 12
<212> DNA
<213> Porcus

<400> 25
agcatctgaa gc 12

<210> 26
<211> 22
<212> DNA
<213> Porcus spp

<400> 26
atggatcctc cattttccaa cc 22

<210> 27
<211> 18
<212> DNA
<213> Porcus spp

<400> 27
ttgtcgacat ctactggc 18

<210> 28
<211> 58
<212> DNA
<213> Porcus spp

<400> 28
ggatcctcac tgtctctcct gatgagatgc gactctcctc tttgcccgtc cgtcctcc 58

<210> 29
<211> 29
<212> DNA
<213> Porcus spp

<400> 29
gaattcatgg ttctgttgcc tctgcagtg 29

<210> 30
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Porcus spp/ovalbumen chimeric peptide

<400> 30
Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly

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  1               5                  10                  15

Arg Ser Phe Asp Gln Ala Thr Trp Thr Leu Arg
             20                  25


<210> 31
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Porcus spp/ovalbumen
      chimeric peptide

<400> 31
Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly
  1               5                  10                  15

Arg Leu Pro Cys His Phe Thr Asn Ser Gln
             20                  25


<210> 32
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Porcus spp/ovalbumen
      chimeric peptide

<400> 32
Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly
  1               5                  10                  15

Arg Lys Gly Pro His Gly Leu Val Pro Ile His Gln Met Ser
             20                  25                  30


<210> 33
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Porcus spp/ovalbumen
      chimeric peptide

<400> 33
Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly
  1               5                  10                  15

Arg Gly Leu Val Pro Ile His Gln Met Ser
             20                  25


<210> 34
<211> 28
<212> PRT
<213> Artificial Sequence
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<220>
<223> Description of Artificial Sequence: Porcus spp/ovalbumen chimeric peptide

<400> 34
Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly
1 5 10 15

Arg Val Gln Ile Lys Asp Lys Gly Ser Tyr Gln Cys
20 25

<210> 35
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Porcus spp/ovalbumen chimeric peptide

<400> 35
Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly
1 5 10 15

Arg Cys Ser Ser Thr Gln Gly Tyr Pro Glu Pro Gln Arg
20 25

<210> 36
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Porcus spp/ovalbumen chimeric peptide

<400> 36
Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly
1 5 10 15

Arg Lys Ser Gln Ala Tyr Phe Asn Glu Thr Gly Glu Leu
20 25

<210> 37
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Porcus spp/ovalbumen chimeric peptide

<400> 37
Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly
1 5 10 15

Arg Ala Ser Leu Lys Ser Gln Ala Tyr Phe Asn Glu Thr

20 25

<210> 38
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Porcus spp/ovalbumen chimeric peptide

<400> 38
Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly
1 5 10 15

Arg Tyr Met Gly Arg Thr Ser Phe Asp Gln Ala Thr Trp Thr
20 25 30

<210> 39
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Porcus spp/ovalbumen chimeric peptide

<400> 39
Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly
1 5 10 15

Arg